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Methods for long-term 17β-estradiol administration to mice

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ABSTRACT

Rodent models constitute a cornerstone in the elucidation of the effects and biological mechanisms of 17β-estradiol. However, a thorough assessment of the methods for long-term administration of 17β-estradiol to mice is lacking. The fact that 17β-estradiol has been demonstrated to exert different effects depending on dose emphasizes the need for validated administration regimens. Therefore, 169 female C57BL/6 mice were ovariectomized and administered 17β-estradiol using one of the two commonly used subcutaneous methods; slow-release pellets (0.18 mg, 60-day release pellets; 0.72 mg, 90-day release pellets) and silastic capsules (with/without convalescence period, silastic laboratory tubing, inner/outer diameter: 1.575/3.175 mm, filled with a 14 mm column of 36 μg 17β-estradiol/mL sesame oil), or a novel peroral method (56 μg 17β-estradiol/day/kg body weight in the hazelnut cream Nutella). Forty animals were used as ovariectomized and intact controls. Serum samples were obtained weekly for five weeks and 17β-estradiol concentrations were measured using radioimmunoassay. The peroral method resulted in steady concentrations within – except on one occasion – the physiological range and the silastic capsules produced predominantly physiological concentrations, although exceeding the range by maximum a factor three during the first three weeks. The 0.18 mg pellet yielded initial concentrations an order of magnitude higher than the physiological range, which then decreased drastically, and the 0.72 mg pellet produced between 18 and 40 times higher concentrations than the physiological range during the entire experiment. The peroral method and silastic capsules described in this article constitute reliable modes of administration of 17β-estradiol, superior to the widely used commercial pellets.

1. Introduction

Estrogens have in recent years been the subject of extensive research and public debate because of their widespread clinical use for contraception and amelioration of post-menopausal symptoms. Rodent models constitute a cornerstone in the elucidation of their effects and detailed biological mechanisms. When using mice, it is common practice to ablate the main source of estrogens by ovariectomy and subsequently administer exogenous 17β-estradiol. The aim of this procedure is to achieve stable 17β-estradiol concentrations that are within the physiological range but lack the natural cyclicity. However, despite the multitude of studies in which these procedures are applied, a thorough assessment of methods for long-term administration of 17β-estradiol to mice has hitherto been lacking. Further, earlier studies in rats have demonstrated that serum concentrations resulting from different administration methods may differ substantially [8,17]. The use of well-known and validated administration regimens is particularly important when studying estrogens, since they have been demonstrated to exert diametrically different effects depending on serum concentrations and dose. One example is the dichotomous effects of estrogens in ischemic stroke. Whereas some studies in rats have shown a neuroprotective effect, others have reported neurotoxicity, which has been suggested to be a dose dependent phenomenon [16,18].

The most frequently used long-term administration methods today are based on subcutaneous administration using slow-release pellets from the company Innovative Research of America (IRA) or silastic capsules. Some studies have attempted to evaluate these by bioassays, e.g. uterine weight and vaginal smears [5], or by measuring the 17β-estradiol concentrations on one single occasion [10]. However, a proper assessment necessitates 17β-estradiol concentration measurements in samples obtained on multiple occasions during the entire evaluation experiment. With one single sample, most often from serum obtained during sacrifice of the animals, it is impossible to estimate variations over time, and thus the temporal hormone profile remains unknown.

Since estrogens are usually administered *per os* to humans, an animal model where the hormone is delivered by this route appears particularly attractive for pharmacokinetic reasons. Even though attempts have been made at peroral administration, e.g. by oral gavage [4,12] and drinking water [7,11], these methods have several shortcomings. Oral gavage requires stressful handling,

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and with estrogens provided in the drinking water it is difficult to control the individual intake of each animal.

The primary aim of the present study was therefore to compare the serum 17β-estradiol concentrations achieved by the commonly used methods for long-term 17β-estradiol-administration to female mice for five weeks. We also characterized, both in short- and long-term perspective, a novel peroral method where 17β-estradiol is administered in the hazelnut cream Nutella. Since there are examples of studies both with [5] and without [1] convalescence periods (washout), a secondary aim was to evaluate these two approaches.

2. Materials and methods

2.1. Animals

One-hundred and sixty-nine female C57BL/6 mice (8–10 weeks, 23 ± 0.09 g) were obtained from Charles River Laboratories (Sulzfeld, Germany). The mice were housed ten in each cage (height: 15 cm, base: 42 × 26 cm) preoperatively, and solitarily during the postoperative period. The room temperature was 21 °C and a 12-h light/dark cycle was maintained. Standard rodent chow (CRM (E); Special Diets Service, Essex, UK) and tap water was provided *ad libitum*. For at least 14 days prior to the experiment, the animals were allowed to habituate to the housing conditions and routine handling by animal technicians. All procedures were conducted in accordance with the National Committee for Animal Research in Sweden and Principles of Laboratory Animal Care (NIH Publication No. 86–23, revised 1985). The protocol was approved by the Local Ethics Committee for Animal Care and Use at Linkoping University.

2.2. Grouping

The animals were randomly assigned into five treatment groups ($n = 20$, except for the peroral group; $n = 34$) subjected to different 17β-estradiol administration methods, one intact control group ($n = 30$) studied in different phases of the estrous cycle (proestrus, diestrus and estrus) and one ovariectomized control group (ovariectomized control group; $n = 10$). Each treatment group was further divided into subgroups A and B from which blood samples were drawn on days 2, 14, 28 and 7, 21, 35, respectively (Fig. 1). Thus, the mice were allowed 14 days of restitution between the samples to avoid anemia, and still it was possible to monitor the 17β-estradiol levels weekly. Ear punching was used for individual identification.

2.3. Ovariectomy and hormone replacement

Fourteen to 21 days before day 0 of the experiment, ovariectomies were performed via the dorsal route in four of the treatment groups (the low-pellet, high-pellet, silastic capsule (with convalescence) and peroral groups), whereas one treatment group (the silastic capsule (without convalescence) group) underwent surgery on day 0.

The low-pellet and high-pellet groups received subcutaneously implanted pellets, 3 mm in diameter, containing 0.18 mg and 0.72 mg 17β-estradiol, respectively (60-day release, SE-121, 0.18 mg/pellet and 90-day release, NE-121, 0.72 mg/pellet; IRA, Sarasota, FL, USA). These doses are frequently used by researchers and have been claimed to establish physiological concentrations of 17β-estradiol in mice [2,6,11,13,19]. A 0.5 cm incision was made in the loose skin of the mouse's neck, and a small pocket was bluntly dissected caudolaterally, in which the pellet was installed using tweezers. The incision was subsequently closed by a suture. During the implantation procedure, strict hygiene was maintained to minimize the risk of infection, and care was taken not to expose the pellets to any solvent or alcohol.

The silastic capsule groups received identical silastic capsules, and thus only differed regarding convalescence time. Twenty mm segments of silastic laboratory tubing (inner/outer diameter: 1.575/3.175 mm, Dow Corning, VWR International, Buffalo Grove, IL, USA) were filled with 17β-estradiol dissolved in sesame oil (36 μg 17β-estradiol/mL; dose based on an unpublished pilot study) and capped with 3 mm pieces of wooden applicator sticks (Birch, length 15 cm, diameter 2 mm; SelefaTrade AB, Spånga, Sweden). This design provided a 14 mm length of tubing through which 17β-estradiol could be released. The capsules were stored overnight in the same solution as inside the capsules, and before implantation, which was performed as with the pellets, the capsules were carefully wiped.

The peroral group received 17β-estradiol perorally in hazelnut cream (Nutella; Ferrero Scandinavia AB, Malmo, Sweden). The mice were kept one in each cage and were served 60 mg Nutella, containing 1.12 μg 17β-estradiol (dose based on an unpublished

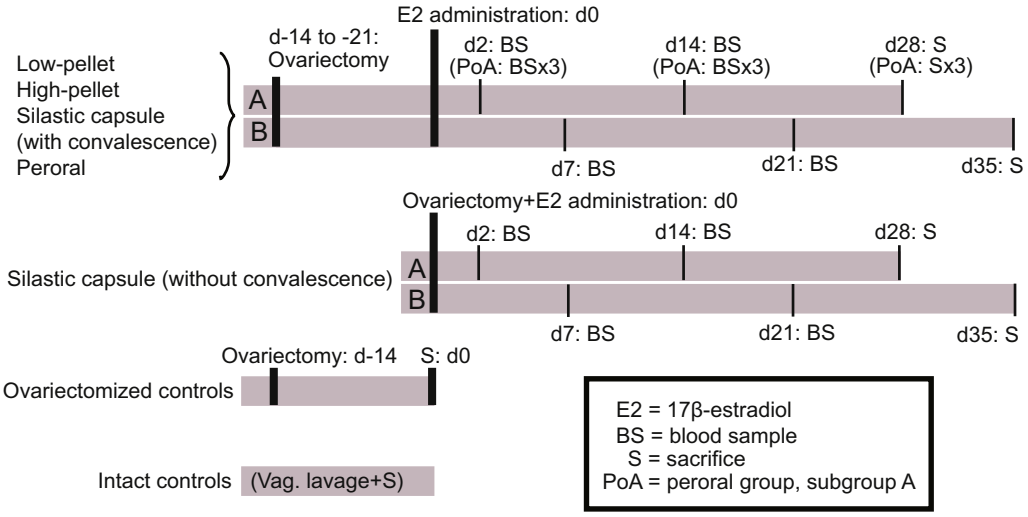

Fig. 1. Timelines for the experimental groups. Four groups (the low-pellet, high-pellet, silastic capsule (with convalescence) and peroral groups) were after ovariectomy and 14–21 days of convalescence administered 17β-oestradiol, and blood samples were subsequently obtained at different time-points. The silastic capsule (without convalescence) group was subjected to the exact same procedures, except without a convalescence period. The ovariectomized control group was sacrificed after ovariectomy and two weeks of convalescence, while the native animals were sacrificed without prior ovariectomy.