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Review

Is there a role for estrogen activity assays? Recombinant cell bioassay for estrogen: Development and applications

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ABSTRACT

There are many questions which cannot be answered without a very sensitive estradiol assay. A recombinant cell bioassay (RCBA) for estradiol was developed in 1994. The sensitivity of the bioassay is 0.02–0.2 pg/ml (0.07–0.7 pmol/L), more than 20 times more sensitive than commercial RIAs and 10 times more sensitive than newer mass spectrometry assays. The RCBA for estradiol opened the door to study low levels of estradiol equivalents (EE) across the physiological spectrum of life from prepubertal children through menopause and across the spectrum from normal physiology, in boys as well as girls, to pathology, including: premature thelarche; estradiol suppression in children treated with GnRH analogues for precocious puberty; aromatase inhibition in boys with growth hormone deficiency; the differences between oral and transdermal routes of estrogen administration in girls with Turner's syndrome; women with breast cancer treated with aromatase inhibitors; and women with urogenital atrophy treated with low dose vaginal estrogen. A bioassay also allows study of endocrine disruptors, like phytoestrogens and other environmental compounds, which are relevant to public health and alternative medicine options. This paper reviews the assay and the last 20 years of applications. A bioassay for estrogen has a role because measuring biological effect is theoretically useful, increasing the understanding of physiology in addition to biochemical levels, giving different information than other assays, and opening the door to measure very low levels of estrogen activity in both humans and the environment.

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1. Introduction

There are many questions that could not previously be answered because of the lack of sensitivity of estradiol (E2) assays. This problem led to the development of a recombinant cell bioassay (RCBA) for estradiol in 1994 [1]. Over the past 20 years, this assay has been used in a wide range of clinical research settings from normal physiology in prepubertal children through menopause, and in pathological states and treatments involving very low levels of estrogen. The present paper reviews the assay development and the last 20 years of applications.

2. Assay and applications

2.1. Estrogen bioassay

The bioassay for E2 uses a strain of *Saccharomyces cerevisiae* that is transformed with two plasmids. One plasmid contains the human estradiol receptor complementary DNA, and the other contains an estradiol response element upstream of the yeast iso-1-cytochrome C promoter fused to LACz, the structural gene for β-galactosidase. The transformed yeast is grown in selective media in the presence of extracted estradiol for 7 h. β-galactosidase activity is measured over time. The sensitivity of the bioassay ranges from 0.02 to 0.2 pg/ml (0.07–0.7 pmol/L). The intraassay and interassay coefficients of variation at 0.2 pg/ml (0.7 pmol/L) range from 10% to 50%. The standard curve is made by weighing estradiol and diluting into ethanol (Fig. 1). The assay is surprisingly specific for E2, with some cross-reactivity with estrogen metabolites. The assay correlates well with a standard RIA in the range measurable by RIA (Fig. 2). The limitations of the assay, and the major reasons why it is still a research assay rather than commercially available, include the expected variability of a bioassay and the extreme sensitivity of the yeast to the presence of estrogen. However, research use of the assay in the following situations shows the importance of continuing to improve methods for measuring both very low levels of E2 as well as estrogen bioactivity. All of the E2 levels reported in the following studies were measured using this RCBA. EE will be used as an abbreviation, since the bioassay more accurately represents estradiol equivalents.

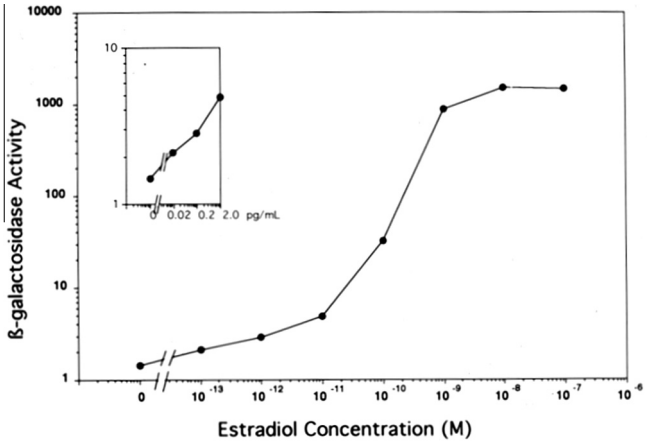

Fig. 1. Dose response curve of B-galactosidase activity vs. concentration of estradiol added to charcoal stripped plasma. Inset shows the low end of the curve.

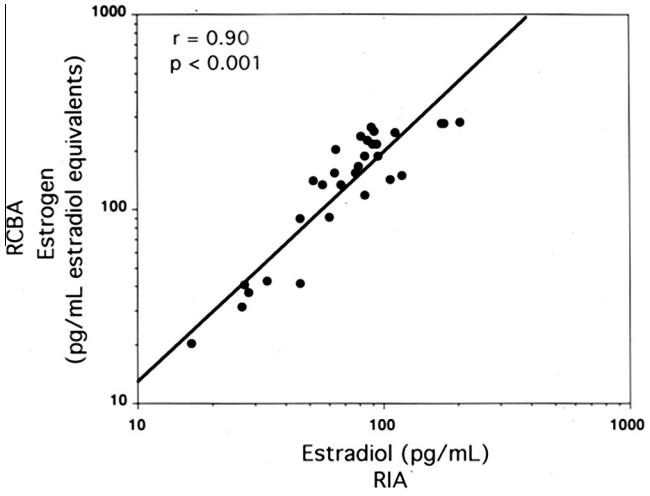

Fig. 2. Linear regression of estrogen levels by RCBA vs. estradiol levels by RIA.

3. Applications

3.1. What are estrogen levels in prepubertal children?

Prior to 1994, Bidlingmaier reported similar E2 levels in prepubertal boys and girls, but levels very close to the detection limit of the RIA used [2]. In 1994, we published much lower EE using the RCBA than previously thought, and levels significantly lower in prepubertal boys than girls [1] (Fig. 3). This is consistent with the differences in bone maturation and prepubertal growth observed between prepubertal girls and boys.

3.2. What are E2 levels in normal boys?

Boys have much lower E2 levels than girls throughout life. We studied 23 normally growing boys and measured hormone levels and growth every 4 months for 5–8 years [3]. EE levels correlate strongly with testosterone levels and with peak growth velocity as boys progress through puberty (Fig. 4). This is consistent with the hypothesis that E2 augments skeletal growth in boys as well as girls.

3.3. Are E2 levels in girls with premature thelarche higher than normal prepubertal girls?

Premature thelarche is the presence of breast tissue in girls prior to the onset of puberty, sometime less than age 8 years. We studied 20 girls with premature thelarche compared to 15 age-matched normal prepubertal girls (mean age 1.4 ± 0.5 year, range 0.5–3.0 year) [4]. EE levels were significantly higher in the girls with premature thelarche (data not shown). This supports the mechanism of premature thelarche being increased E2 levels rather than increased sensitivity of breast tissue to estrogens.

3.4. What are E2 levels in children with precocious puberty on treatment?

Precocious puberty is defined as the onset of hypothalamic-pituitary-gonadal axis activation prior to age 8 years in girls or