Bioidentical hormones used in Hormone Replacement Therapy: Implications for breast cancer

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Introduction:

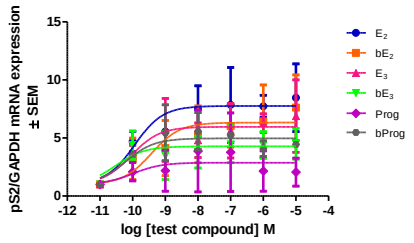
Hot flashes, night sweats and bone loss are some of the symptoms experienced by post-menopausal women, and are caused by decreased endogenous estrogen production. Conventional hormone replacement therapy (HRT) is administered to post-menopausal women as estrogen alone or as an estrogen-progestogen combination to alleviate these symptoms. However, severe side-effects including increased risk of breast cancer are associated with HRT use. This has led many women to seek possible safer, "natural" alternatives such as bioidentical hormones; compounds chemically modified from a natural precursor to have the exact structure of the endogenous human hormones. Considering that steroid hormones can elicit their effects via steroid receptors, including the estrogen receptors (ERs), and that the ERs have been implicated in breast cancer, we investigated the roles that bioidentical hormones may play in breast cancer by investigating their effects on gene expression and proliferation in a breast cancer cell line and characterising their activity via the ER subtypes, ERα and ERβ. The hormones investigated include natural and bioidentical estradiol (E_2, bE_2), estriol (E_3, bE_3) and progesterone (Prog, bProg).

Questions:

1. Do the bioidentical hormones have similar effects on (a) gene expression and (b) proliferation to the natural hormones in a breast cancer cell line?
2. Do the ER subtypes have similar binding affinities for the bioidentical hormones as the natural hormones?
3. Are the relative agonist potencies of the bioidentical and natural hormones similar for transactivation via the ER subtypes?

Results:

1a The bioidentical and natural hormones have similar relative agonist potencies for transactivation of an endogenous ERE containing gene

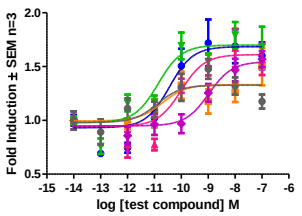


Summary of potencies and efficacies for induction of pS2 gene expression

	Efficacy	Potency (nM)
E_2	7.76 ± 0.76	0.11 ± 0.054
bE_2	6.33 ± 1.02	0.38 ± 0.41
E_3	5.96 ± 0.80	0.09 ± 0.023
bE_3	4.28 ± 0.82	0.031 ± 0.0015
Prog	2.88 ± 0.82	0.11 ± 0.51
bProg	4.97 ± 0.52	0.063 ± 0.0023

The MCF-7 BUS cell line was incubated with either 0.1% (v/v) ethanol (EtOH) (solvent control) or increasing concentrations of test compounds for 24 hours. Total RNA was isolated and real-time quantitative PCR (qPCR) was performed to determine the mRNA expression levels of Trefoil Factor 1 (pS2), using GAPDH as the internal standard. pS2 is an estrogen responsive gene that is used as a marker of breast cancer progression.

1b The bioidentical hormones induce similar proliferation of a breast cancer cell line as the natural hormones.

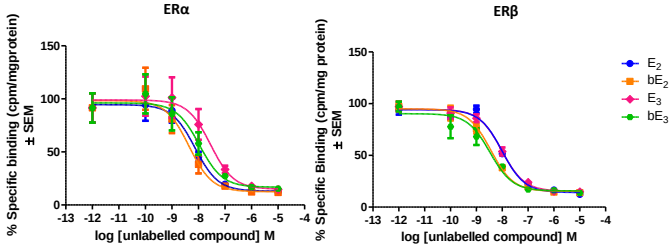


Summary of potencies and efficacies for induction of proliferation

	Efficacy	Potency (nM)
E_2	1.53 ± 0.18	0.033 ± 0.0027
bE_2	1.39 ± 0.21	0.072 ± 0.058
E_3	1.64 ± 0.20	0.16 ± 0.034
bE_3	1.68 ± 0.18	0.025 ± 0.0082
Prog	1.47 ± 0.12	0.40 ± 0.28
bProg	1.36 ± 0.075	0.038 ± 0.027

Cells were treated with either 0.1% (v/v) EtOH (solvent control) or increasing concentrations of the test compounds for 44 hours after which the colorimetric MTT assay was used to measure cell proliferation. Results are expressed as fold induction relative to solvent. The figure is a representation of experimental data.

2 The ER subtypes have similar binding affinities for the natural and bioidentical estrogens, while Prog and bProg do not bind

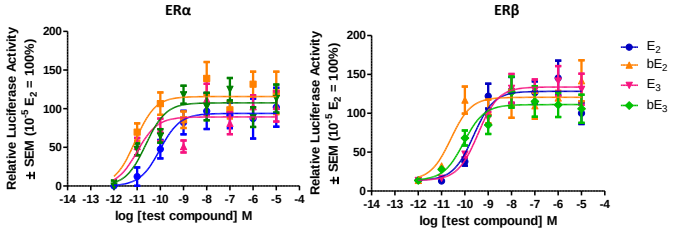


Summary of binding affinities (K_d or K_i) of the ligands for ERα and ERβ

	E_2	bE_2	E_3	bE_3
ERα	0.36 ± 0.12	0.084 ± 0.011	0.45 ± 0.039	0.31 ± 0.047
ERβ	2.48 ± 0.60	0.85 ± 0.11	2.29± 0.62	0.99± 0.29

COS-1 cells transfected with cDNA expression vectors for the human ERα or ERβ respectively were incubated with 10 nM or 20 nM [^{3}H]-E_2 in the absence (0.1% (v/v) EtOH (total binding)) or presence of increasing concentrations of unlabelled test compounds (non-specific binding) for 4 hours. Counts per minute (cpm) were determined and normalized to protein concentrations and the total binding was set as 100%. Statistical analysis of the binding affinities for all compounds indicated no significant difference.

3 The bioidentical and natural estrogens have similar relative agonist potencies for transactivation via ERα but not ERβ



Summary of potencies and efficacies for agonist activity via an ERE

	E_2	bE_2	E_3	bE_3
Efficacy ERα	2.21 ± 0.50	2.53 ± 0.34	2.18 ± 0.28	2.40 ± 0.32
Potency ERα (nM)	0.16 ± 0.010	0.0069 ± 0.015	0.34 ± 0.33	0.045 ± 0.0.036
Efficacy ERβ	9.48 ± 2.24	10.19 ± 2.21	9.81 ± 2.23	8.264 ± 2.24
Potency ERβ (nM)	0.24 ± 0.061	0.044 ± 0.010	0.40 ± 0.13	0.098 ± 0.0016

COS-1 cells were transiently transfected with cDNA expression vectors for the human ERα or ERβ and an ERE-containing promoter-luciferase reporter construct and incubated with either 0.1% (v/v) EtOH (solvent control) or increasing concentrations of test compounds that bind the ER subtypes for 24 hours.

Discussion and Conclusion:

Results show that the bioidentical and natural hormones have similar :

1. effects on mRNA expression of the pS2 gene
2. effects on breast cancer cell line proliferation
3. binding affinities for the ER subtypes
4. relative agonist potencies for transactivation via ERα but not ERβ.

Funding