Fortune Kohen

Batya Gayer
Tikva Kulig
Gil Mor

Anti-idiotypes and isoflavones as probes for estrogen action

Department of Biological Regulation

Tel. 972 8 934 2763 **Fax.** 972 8 934 4116
E-mail: fortune.kohen@weizmann.ac.il

Rationale

Estradiol, a steroid hormone, regulates the growth, differentiation and function of diverse tissues, both within and outside the reproductive system. The hormone's relatively slow biological effects are mediated via two nuclear estrogen receptors, ER α and ER β, present mainly in the reproductive system, whereas the fast non-genomic effects are mediated via a membranal binder whose structure is not yet elucidated. The two ER isoforms exhibit distinct tissue distribution patterns and differ in their ligand binding ability and transactivational properties. A variety of chemicals with no obvious structural similarity bind to the C-terminal ligand binding domain (LBD) of ER and function as pure agonist (e.g. 17β-estradiol, see Fig. 1) whereas others such as 4-OH-tamoxifen, genistein (see Fig. 1), known as selective estrogen modulators (SERMs), function as agonist or antagonist depending on the tissue and promotor context. The 3D structures of human ER α-LBD in complex with estradiol, with tamoxifen, and with raloxifene, and of ER β-LBD in the presence of genistein and of raloxifene have been determined. It has been shown that the ability of ER ligands to act as agonists or antagonists can be related to the position adopted by helix 12 (H12). In the presence of agonists, H12 is oriented across the cavity so that the bound ligand is completely buried within the core of LBD. In contrast, the large side-chain substituent of tamoxifen, cannot be accommodated within the confines of the binding cavity. Instead, the substitutent protrudes from the binding cavity and prevents the correct alignment of H12.

Because of the occurrence of both beneficial and unwanted effects during hormone replacement therapy and breast cancer prevention and treatment, the key to improvement of drug therapy is the development of SERM's with better tissue selectivity. Due to the diversity of estrogen action our current interest focuses in two areas: (i) development of novel probes such as anti-idiotypes, that can act as a surrogate ligand for estrogen and (ii) rational design of new SERMs based on the structure of the isoflavone genistein.

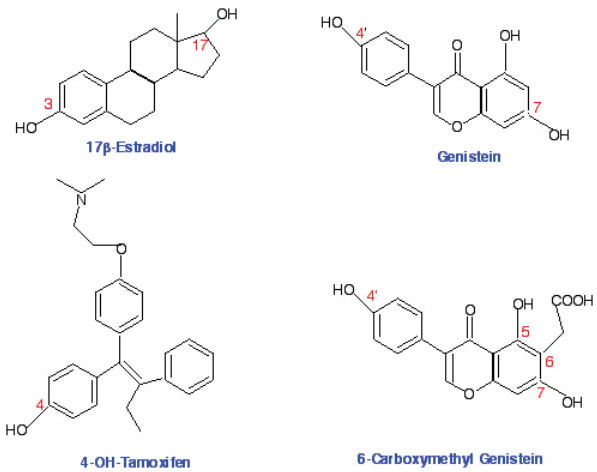

Fig. 1 *Estrogenic compounds exhibit a variety of molecular structures.*

Recent findings and comments

The anti-idiotypic antibody, clone 1D5, raised against anti-estradiol antibody, clone 2F9, was used as a probe in identifying the estrogen receptor α in thymocytes and monocytes, indicating a cross-talk between the immune and reproductive systems.

The anti-idiotypic antibody, clone 1D5, mimics the genomic and non-genomic actions of estradiol in vivo in rat tissues and in vitro in estrogen responsive cell lines (e.g. skeletal, endothelial etc). The transport mechanism responsible for the genomic effects (e.g. creatine kinase stimulation) of the anti-idiotypic antibody is unknown.

Since the anti-idiotypic antibody mimics the actions of estradiol, it can be suggested that there may be a structural homology between the binding site of the primary anti-estradiol antibody and ER.

A novel genistein (G) derivative, 6-carboxymethyl genistein (CG) (Fig. 1) showed estrogenic activity like G in vitro and in vivo. On the other hand G and CG differed in the following

parameters: (i) only CG displayed mixed agonist antagonist activity for ER alpha in transactivation assays and (ii) only CG was capable of attenuating estrogen induced vascular smooth muscle cells proliferation and of inhibiting estrogen induced creatine kinase (CK) stimulation in rat tissues. On the other hand, only G caused an increase in stimulating the CK specific activity in the uterus. In comparison to the SERM raloxifene CG is as effective at 5 to 10 times lower concentrations, showing the same selectivity profile as RAL in blocking the CK response to estrogen in tissues derived from both immature and ovariectomized female rats. These results suggest that CG can be considered a novel SERM with unique properties on the bone, uterus and vasculature.

Modeling of CG in the LBD of ER α (Fig. 2) indicates that the carboxy group in CG perturbs the end of Helix 11 by eliciting a severe backbone change for Leu 525, and inducing a conformational change which could position Helix 12 in an antagonist conformation. This model supports the experimental findings that 6-carboxymethyl genistein can act as a SERM in the presence of ER α.

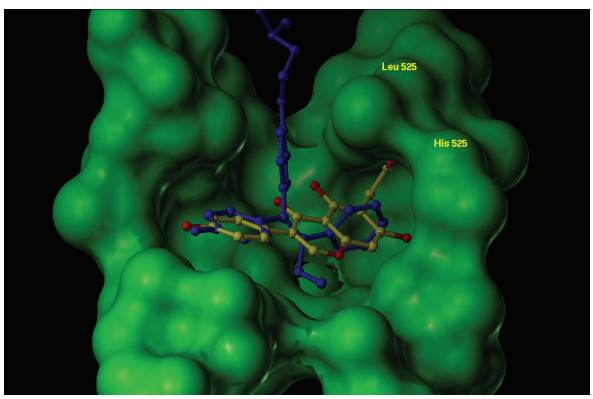

Fig. 2 *Computer modeling of the ligand binding domain of estrogen receptor alpha complexed with 6-carboxymethyl genistein (CG) or tamoxifen. Color scheme: yellow for CG, blue for tamoxifen, red for oxygen atoms and green for estrogen receptor.*

Selected Publications

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Acknowledgements

We thank Drs. D. Somjen, N. Stern from the Institute of Endocrinology, Tel-Aviv Souransky Medical Center and the Seckler Faculty of Medicine, Tel-Aviv University and Dr. L. Toldo from Department of Bio and Chemoinformatics. Merck KGaA, Darmstadt, Germany for permission to quote collaborative work and to the Ministry of Health, Jerusalem for funding part of this work.