Studying potential endocrine disrupting chemicals: comparison of yeast and human cell-based in vitro assays

The production of chemicals for agricultural and industrial use, cosmetics or food additives is steadily increasing. To protect the environment and human health against the potential risks arising from these man-made chemicals, the authorities introduced several programs to promote adequate substance-based hazard and risk characterizations. Such chemicals may cause harmful effects by disturbing the hormonal system. These so-called endocrine disrupting chemicals (EDCs) can mimic or block the action of endogenous hormones on their corresponding receptors, disrupt their synthesis, metabolism, transport or underlying signaling pathways. Sex steroid hormones (androgens, estrogens) orchestrate in particular developmental and reproductive functions, whereas glucocorticoids (especially cortisol) essentially regulate the immune system, blood pressure, energy metabolism and developmental aspects. Disruption of the endocrine system has been associated with developmental and reproductive malfunctions, allergies, diabetes, cardiovascular diseases, and cancer.

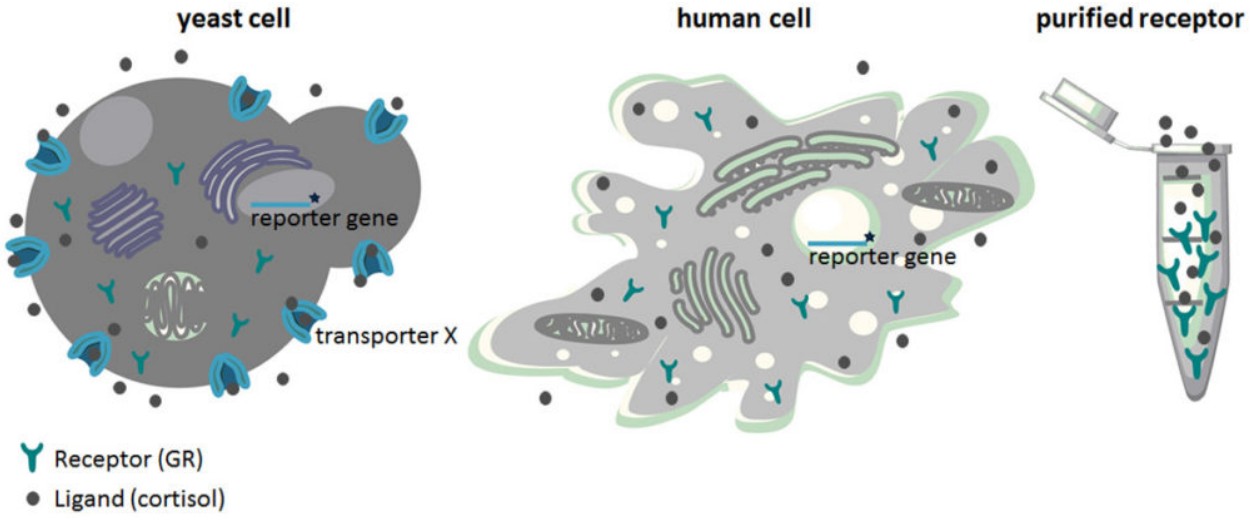

Fig. 1. Schematic representation of a yeast and a human reporter gene cell and the purified receptor in a test tube. Due to a transporter X on the yeast cell membrane, the yeast cell is able to export glucocorticoids, thereby limiting the ligand accessibility to the GR. This alters the assay sensitivity and could lead to false positive or false negative results. In comparison, the human cell is lacking this transporter X. However, the only test system with free ligand accessibility represents the purified receptor in a test tube. Therefore, an adequate evaluation of chemicals acting on steroid hormone receptors should include a combination of cell-based and cell-free assays.

In vitro testing systems play a crucial role during the safety assessment of EDCs and gained even more importance since chemicals used in body care products can no longer be tested in animals.

These *in vitro* studies are conducted with microorganisms or cellular components in an artificial system separated from their natural environment. Human cell-based assays are widely used to study EDC action on hormone receptors, whereby receptor-mediated reporter genes are used as receptor activation read-out. A cost-effective alternative are yeast reporter cell assays. However, each *in vitro* testing system has its limitations, which needs to be considered for data analysis. Yeast androgen (YAS) and estrogen screen (YES) show limited uptake of certain compounds through the yeast cell membrane and may not sufficiently allow discriminating between compounds activating or suppressing the receptor activity.

The extensively applied flame retardant tetrabromobisphenol A (TBBPA) was previously reported to strongly inhibit the activity of the glucocorticoid receptor (GR) and to a lesser extent the androgen receptor (AR) in yeast reporter gene assays. Regarding the limitations of these systems, the current study evaluated this observation in a human cell-based assay. TBBPA did not stimulate nor suppress GR activity. To exclude limitations due to different cellular uptake of TBBPA by yeast and human cells, receptor binding assays were conducted under cell-free conditions with direct access of the compound to the receptor. Nevertheless, this experiment allows only conclusions on the affinity of a substance to bind to a receptor but not its ability to activate or inhibit. In this regard, TBBPA did not affect the ligand binding to the GR. Computer simulations with TBBPA and GR supported this finding. Thus, TBBPA does no directly disturb GR function. However, inhibition of the AR could be confirmed in the human cell-based and cell-free systems, although the effect was much less pronounced.

The discrepancy to the earlier report may be due to limitations of the yeast assay. GR yeast assays are known for their low sensitivity, probably due to a yeast specific transporter exporting glucocorticoids from the cell. By upregulating the expression of this transporter TBBPA might indirectly affect the glucocorticoid concentration in the yeast cell and therefore lead to false positive results. Thus, it is highly important to be aware of the limitations of each *in vitro* testing system to avoid false positive or false negative results. Many studies applied TBBPA concentrations far above those found in the human body. However, appropriate risk assessment should always include the evaluation of the substance at the concentration found in human. TBBPA is rapidly degraded and only detected at very low concentrations in human. Thus, TBBPA unlikely causes adverse effects by disrupting GR or AR action. The current study highlights the use of a combination of cell-based and cell-free assays for the evaluation of chemicals on steroid hormone receptors.

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