THE IMPACT OF CB-153 AND *p,p'*-DDE ON ANDROGEN RECEPTOR FUNCTION

Björk C[1], Nenonen H[1], Giwercman A[1,2], Bergman Å[3], Rylander L[4], Lundberg Giwercman Y[1]

[1]Department of Clinical Sciences, Molecular Genetic Reproductive Medicine, Lund University, Malmö, Sweden; [2]Reproductive Medical Centre, Malmö University Hospital, Malmö, Sweden; [3]Department of Environmental Chemistry, Stockholm University, Stockholm, Sweden; [4]Division of Occupational and Environmental Medicine, Lund University, Lund, Sweden.

Abstract

Reports regarding hampered male reproductive function due to exposure of exogenous agents have been published over the last decades. Agents of concern include persistent organohalogen pollutants (POP) that could modulate endocrine signalling pathways by interfering with natural hormones such as androgens or oestrogens by activation or inhibition of estrogen receptor and androgen receptor (AR) induced responses. All androgen actions are mediated through the AR, which contains a polymorphic glutamine repeat commonly referred to as the CAG repeat. High POP exposure in combination with a short CAG repeat (<20) has been associated with significantly lower sperm concentration. Our aim was to investigate if a mix of the two POP markers *p,p´*-DDE and CB-153, could influence the AR activity in a CAG repeat length depending manner. The ability of three different CAG lengths (16, 22 and 28) to activate a reporter gene, together with 10 nM 5α-dihydrotestosterone (DHT), CB-153 and *p,p´*-DDE was tested *in vitro*. In the presence of CB-153 and *p,p´*-DDE, a median 26% decreased activity was observed for AR CAG28 (p=0.03), as compared to AR CAG28 with no CB-153 and *p,p´*-DDE added. This indicates that certain genotypes of the AR could be linked to a higher sensitivity to POP exposure.

Introduction

Reports of declining semen quality in parallel with a rise in testicular cancer and congenital malformations have been published[1-3]. These disorders have together been called Testicular Dysgenesis Syndrome (TDS) and are most likely initiated during early foetal development[4]. TDS may be caused by external factors such as endocrine disruptors in combination with genetic predisposition. Although the production of persistent organohalogen pollutants (POPs), like PCBs and DDT, has been banned for 30 years in most countries, the compounds are still detected in animals and humans all over the world[5]. Previous studies have shown their estrogenic, anti-estrogenic and anti-androgenic effects both *in vitro* and *in vivo*[6-9].

Androgens, together with a functioning androgen receptor (AR), are essential for normal development of male reproductive organs in the foetus, for puberty and spermatogenesis thereafter[10]. The AR contains a polymorphic glutamine (CAG) repeat, which is varying between individuals and populations of different ethnic origin, with African American having on average fewer CAG repeats than Caucasians and Far-Eastern Asians[11]. In the general Swedish population the median CAG repeat length is 22 amino acids, ranging from 12 to 30 repetitions, whereas the Inuit on Greenland, who are genetically related to the Asians, have a median CAG repeat length of 23[12] . The Inuit is one of the most POP exposed populations on earth because of the diet of sea mammals like seal and whale.

In a previous study on three different populations in Europe and the Inuit on Greenland, high POP exposure in combination with a short AR CAG repeat (<20) was associated with 35% lower sperm concentration and a 42% lower total sperm counts[13]. Our objective was therefore to *in vitro* investigate if a mix of CB-153 (2,2',4,4',5,5'-hexachlorobiphenyl) and *p,p'*-DDE, both markers for POP exposure, could influence AR activity when varying the CAG repeat length.

Materials and methods

An AR vector harbouring one of three different repeat lengths of the CAG repeat (16, 22 and 28), was co-transfected into COS-1 cells, which do not express endogenous AR, together with a firefly-luciferase androgen responsive reporter plasmid and a renilla transfection efficiency control plasmid. This was done in the absence or presence of 10 nM 5α-dihydrotestosterone (DHT) together with a mix of the two POP markers, *p,p´*-DDE and CB-153 in concentrations corresponding to what was found in the study on three populations in Europe and the Inuit on Greenland[14]. The luciferase and renilla activities were measured by a Dual-Luciferase reporter system. Total protein content was analyzed by Quick start Bradford protein assay. Six experiment repeats where done in duplicates.

Wilcoxon non-parametric paired test was used to compare the difference in transactivating capacity of each AR construct, correlated to total protein, in the absence or presence of pollutant.

Results and discussion

The AR containing CAG22, untreated with POP, was set as reference (100%) and other constructs compared to this. Both CAG16 and CAG28 showed lower activity than CAG22 (mean 54% and 47%, respectively (Fig. 1).

When the CB-153 and *p,p'*-DDE mixture was added to the cell-reporter system, a median decrease in activity for all lengths was noted compared to untreated cells, (CAG16 14%, CAG22 6% and CAG28 26%), where the decrease for CAG28 was statistically significant (p=0.03) (Fig 2).

With respect to the ability to drive a reporter gene, our main finding was that an AR with a long CAG repeat length seems to be the most resistant variant to the effect of CB-153 and p,p'-DDE within physiological concentrations. This did not support our hypothesis from the *in vivo* study, where a short AR CAG repeat length was most susceptible to the effect of POPs. In previous studies where mixtures of anti-androgens like for example PCBs and phthalates or a mixture of PCBs were assessed adverse effects on semen parameters and reproductive hormone levels *in vitro* and *in* vivo were noticed[15-16]. It is an important issue to further investigate the risks of POPs, together with genetic susceptibility regarding male fertility, with respect to future human risk assessments of environmental pollutants.

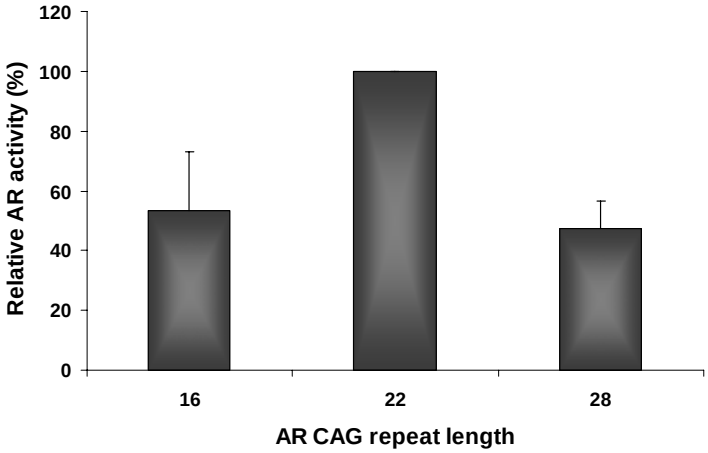

Figure 1. The relative activities of the AR harbouring CAG repeat lengths of 16, 22 and 28 with 10 nM DHT. The bars represent the mean activity with corresponding standard deviations from six experiment repeats in duplicates.

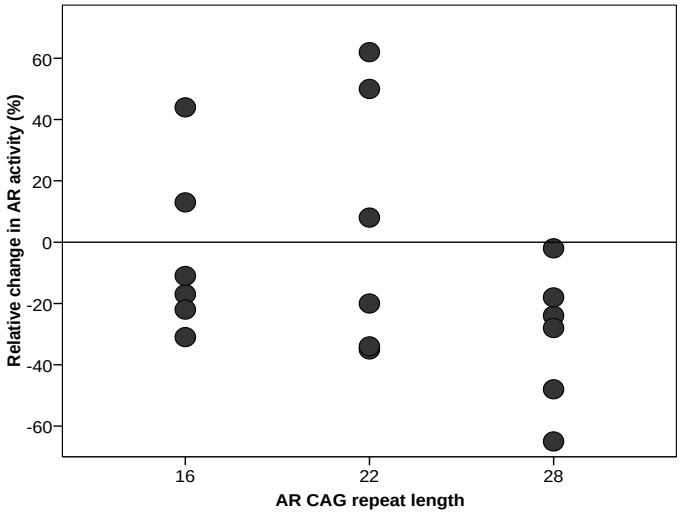

Figure 2. The relative change in activity for ARs carrying 16, 22 or 28 CAG repeats, after addition of CB-153 and p,p'-DDE and 10 nM DHT. The dots represent the relative increase or decrease in activity of the respective AR CAG repeat length, after addition of the pollutants for each experiment in duplicates. A change of 0 % symbolizes no effect.

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