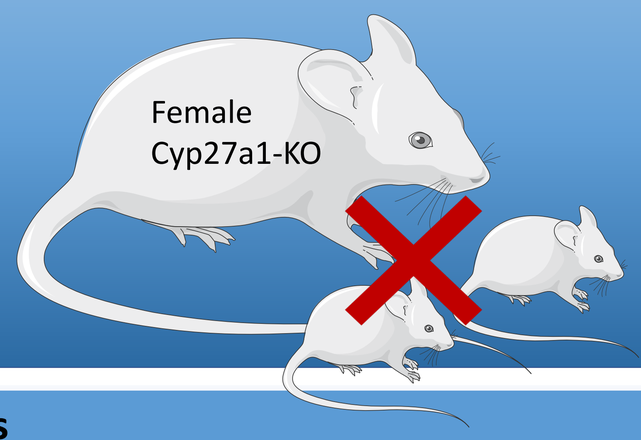
Role of steroids in the infertility of Cyp27a1 KO and Cyp27a1/ ApoE DKO female mice

Flück, Janine, BMA17-20A

College of Higher Education
Educational Program for Biomedical Scientists

University of Bern, Department for BioMedical Research: Nephrology and Hypertension



1. Abstract

Female Cyp27a1 knock out (KO) mice and female Cyp27a1/ApoE-DKO mice are infertile. The aim of this thesis is to find out whether a change in the steroid production of female Cyp27a1-KO and female DKO mice leads to infertility. Hence, steroids have been extracted from the three main steroid synthesis organs (adrenal glands, ovaries and testis) and were measured on a liquid-chromatography mass-spectrometer. Steroids of male and female mice have been compared between the genotypes Wild type (WT) and Cyp27a1-KO and the genotypes ApoE-KO and DKO. These comparisons have been made in both, a normal standard diet and in a high fat western diet.

These comparisons showed in all organs and diets some changes in the steroids. However, despite many significant differences in the individual comparisons, there was no steroid found, which could be responsible for the infertility of Cyp27a1-KO and DKO female mice.

2. Introduction

Gen Cyp27a1:

- Encodes the enzyme sterol 27-hydroxylase (Cyp27a1).
- Function of the enzyme Cyp27a1: converts cholesterol to bile acids, enables the reverse cholesterol transport to the liver [1] and influences the intracellular progesterone regulation [2] and availability of glucocorticoids [3].

Cyp27a1-KO mice have changes in the bile acid metabolism [1], show enlarged liver and adrenal glands, have altered fat levels and **females are infertile** [4].

Apolipoprotein E (ApoE):

- Plays a central role in lipid metabolism, transports lipids among cells of different organs and is a ligand of cell-surface lipoprotein receptors. ApoE is a key regulator of lipid clearance in plasma. [5]
- Different tissues - mainly the liver - synthesize ApoE. [5]

ApoE-KO mice have an imbalance of cholesterol through the deficiency of ApoE expression. With a less effective lipoprotein clearance ApoE-KO mice develop hypercholesterolemia, atherosclerotic lesions and hyper- and dyslipoproteinemia. [6]

Steroid hormones are classified in five groups: mineralocorticoids, glucocorticoids, androgens, estrogens and progestins. They are mainly synthesized in the adrenal gland and the gonads. Androgens, estrogens and progestins play an essential role in the development of secondary sexual characteristics, fertilization and pregnancy. [7]

Mice were fed with either a standard diet (SD) or a challenging high fat western diet (WD).

3. Aim/ Leading Questions

Find out the problem in the steroid production which causes infertility in Cyp27a1-KO and DKO (Cyp27a1$^{-/-}$/ApoE$^{-/-}$) female mice.

Question 1: Are Cyp27a1-KO and DKO (Cyp27a1$^{-/-}$/ApoE$^{-/-}$) females infertile because of a problem in the steroid production in ovaries? (challenged or not with western diet)

Question 2: What is the production of steroids in Cyp27a1-KO and DKO (Cyp27a1$^{-/-}$/ApoE$^{-/-}$) males - what is the effect of western diet?

Question 3: Is the increased size of adrenal glands in Cyp27a1-KO and DKO (Cyp27a1$^{-/-}$/ApoE$^{-/-}$) mice causing a change in steroid production? If yes, does it also explain the inability of females of this genotype to produce viable pups? (challenged or not with western diet)

4. Material and Methods

Female and male C57BL/6 mice with the following genotypes were used: Wild type, ApoE-KO, Cyp27a1-KO and Cyp27a1/ ApoE-DKO. The mice were fed with a regular standard diet or a western diet.

To the organs (adrenal gland, ovary and testis) an internal standard was added and then they were homogenized with bead tubes. The steroids were then extracted using dichloromethane and methanol. After preparation, the samples were measured on an Orbitrap Mass spectrometer.

5. Results

Depending on the organ, different steroids have been detected.

Ovaries: pregnenolone, progesterone, 11-DOC, corticosterone, 17OH-progestrone, androsterone and testosterone (Tested samples: SD: 24; WD: 25)

Testis: pregnenolone, progesterone, 11-DOC, corticosterone, 17OH-progesterone, 11-deoxycortisol, DHEA, androstenedione, testosterone and DHT (Tested samples: SD: 35; WD: 20)

Adrenal gland: pregnenolone, progesterone, 11-DOC, corticosterone and aldosterone (Tested samples: SD: 60; WD: 48)

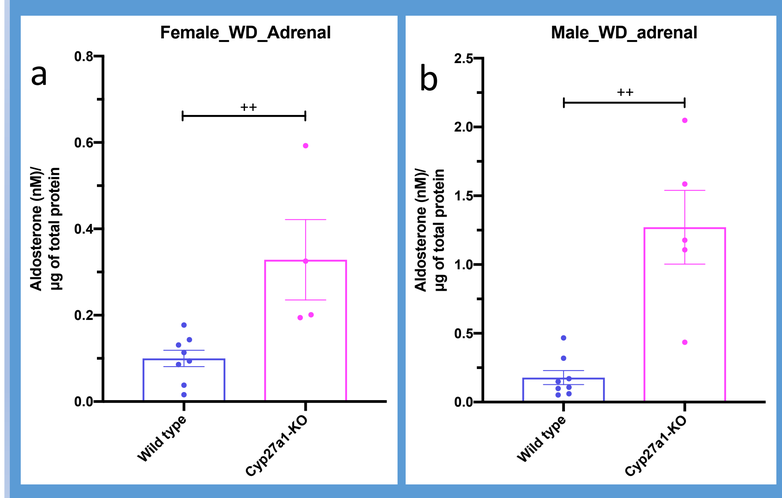


Figure 5.1: Aldosterone production in female (a) and male (b) adrenal glands. Comparison between Cyp27a1-KO and WT. Significant difference is given with **p ≤ 0.0002 (Flück, 2020)

In all three organs, the Cyp27a1-KO group was then compared to the WT control group and the DKO group to the ApoE-KO control group. The comparisons were made in SD and WD. An example is shown in Figure 5.1.

6. Discussion

Question 1: In the ovaries, only the steroid 11-DOC in the group WD - DKO compared to ApoE - showed a significant decrease. Otherwise, no significant difference could be detected. Therefore, the steroid production in the ovaries is not the cause of infertility.

Question 2: In the testis, only the steroid DHEA in the group SD - Cyp27a1-KO compared to WT - showed a significant increase. Else, no significant difference could be detected. Concluding, the steroid production in the testis is not the cause of female infertility.

Question 3: Although some changes in the adrenal glands of both, females and males, no steroid could be identified as the cause for the infertility of females. However, an unexpected upregulation of aldosterone was observed in both gender of the Cyp27a1-KO group, when the mice were fed with WD.

Conclusion: **The detected steroids have no influence on the infertility of female Cyp27a1-KO and DKO mice.** Yet, it was not possible to measure the two essential steroids estrone and estradiol. Hence, it remains possible that a change in the steroid production in the ovaries is responsible for the infertility.

Since these genotypes are used to study arteriosclerosis, the elevated aldosterone in Cyp27a1-KO mice is of great interest as it could affect the blood pressure.

References

[1] Dubrac, S., Lear, S.R., Ananthanarayanan, M., Balasubramaniyan, N., Bollineni, J., Shefer, S., Hyogo, H., Cohen, D.E., Blanche, P.J., Krauss, R.M., Batta, A.K., Salen, G., Suchy, F.J., Maeda, N. and Erickson, S.K. (2005). Role of Cyp27A in cholesterol and bile acid metabolism. *Journal of lipid research*, *46(1)*, 76-85. Retrieved https://www.jlr.org/content/46/1/76.long

[2] Escher, G., V.geli, I., Escher R., Tuckey, R.C., Erickson, S., Krozowski, Z. and Frey, F.J. (2009). Role of Cyp27a1 in progesterone metabolism in vitro and in vivo. *American Journal of Physiology Endocrinology and Metabolism*, *297(4)*, E949-E955. Retrieved https://journals.physiology.org/doi/full/10.1152/ajpendo.00298.2009?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed

[3] Vögeli, I. Jung, H.H., Dick, B., Erickson, S.K., Escher, R., Funder, J.W., Frey, F.J. and Escher, G. (2013). Evidence for a role of sterol 27-hydroxylase in glucocorticoid metabolism in vivo. *Journal of Endocrinology*, *219(2)*, 119-129. Retrieved https://joe.bioscientifica.com/view/journals/joe/219/2/119.xml

[4] Repa, J.J., Lund, E.G., Horton, J.D., Leitersdorf, E., Russel, D.W., Dietschy J.M. and Turley, S.D. (2000). Disruption of the sterol 27-hydroxylase gene in mice results in hepatomegaly and hypertriglyceridemia. *The Journal of Biological Chemistry, 275(50)*, 39685-39692. Retrieved https://www.jbc.org/content/275/50/39685.long

[5] Huang, Y. and Mahley, R.W. (2014). Apolipoprotein E: Structure and Function in lipid metabolism, neurobiology, and Alzheimer's diseases. *Neurobiology of disease*, *72(Pt A)*, 3-12. Retrieved https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4253862/

[6] Sasso, G.L., Schlager, W.K., Bou., S., Veljkovic, E., Peitsch, M.C. and Hoeng, J. (2016). The Apoe-/- mouse model: a suitable model to study cardiovascular and respiratory diseases in the context of cigarette smoke exposure and harm reduction. *Journal of Translational Medicine*, *14(1)*, 146. Retrieved https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4875735/

[7] Holst, J.P., Soldin, O.P., Guo, T., and Soldin, S.J. (2004) Steroid hormones: relevance and measurement in the clinical laboratory. *Clinics in laboratory medicine*, *24(1)*, 105-118. Retrieved https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3636985/

Figures

Cover picture: Smart Servier Medical Art. General Items. Retrieved on 27.01.2020 at https://smart.servier.com/image-set-download/

Figure 5.1: Flück, J. (2020). *Steroid in female and male adrenal glands of Cyp27a1-KO*. Bern: Inselspital