DO PUNICALAGINS HAVE POSSIBLE IMPACT ON SECRETION OF STEROID HORMONES BY PORCINE OVARIAN GRANULOSA CELLS?

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INTRODUCTION

Punicalagins are ellagitannins in which gallic and ellagic acid are linked to a glucose molecule (Cerdá et al. 2003). Punicalagin has two isomeric forms in pomegranate: α and β. Chemical name of punicalin is 2,3-(S,S)-hexahydroxydiphenoyl-4,6-(S,S)-gallagyl-D-glucose (Tyagi et al. 2012). Punicalagins are phenolic compounds which are responsible for the main antioxidant activity of pomegranate (Punica granatum, Punicaceae) (Syed et al. 2007). Punicalagin levels are widely variable in pomegranate juice and can range from as low as 0.014-1.5g. (Syed et al. 2007). Pomegranate peel and pulps have a higher total phenolic and antioxidant activity than juice (Gílóžekéi et al. 2011; Eifaleh et al. 2011).

Punicalagin is a large molecule and knowledge about the fate of ellagitannins (include punicalagins) in human or animals is very sparse. In a recent study by Cerdá et al. (2003) the metabolism of punicalagins was described in rat. Punicalagins or metabolites of punicalagins were detected in faeces, urine and plasma. Native punicalagins were identified in plasma and urine, but at a very low concentration. The main metabolites present in biological fluids are those derived from punicalagins by hydrolysis and future conjugation. Rat microflora is able to metabolise ellagic acid derivatives to produce 6H-dibenzo[b,d]pyran-6-onemetabolites (uroolithins) (Cerdá et al. 2003). Punicalagins were metabolized and/or absorbed. This means that most of the ingested punicalagins have to be transformed to known (punicalin, ellagic acid, gallic acid) or unknown metabolites or accumulated in tissue (Cerdá et al. 2003). This in vitro study was focused on the secretion of steroid hormones, progesterone and 17β-estradiol, by porcine ovarian granulosa cells after punicalagin administration.

MATERIAL AND METHODS

Material

Ovaries (n=12 per experiment) of Slovakian White gilts were obtained from healthy animals without visible abnormalities. All experimental animals are kept under standard conditions at slaughterhouse in Myjava. Ovaries were transported to the laboratory at 4°C and washed in sterile physiological solution.

Isolation of granulosa cells

Ovarian granulosa cells were used in this in vitro study. The suspension of the cells was centrifuged for 10 min at 200xg (to divide the follicular liquid from granulosa cells), washed in sterile DMEM/F12 1:1 medium with 10% fetal bovine serum and 1% antibiotic–antimycotic solution (Gozlekci et al. 2011). The total cells, vital and death cells were assessed using a haemocytometer of porcine ovarian granulosa cells was found.

Viability of granulosa cells

Viability of granulosa cells was assessed by trypan blue solution (0.4%). A reference sample (500 μl) were mixed and incubated 5 min at room temperature. The total cells, vital and death cells were counted using a haemocytometer from minimum 10 fields and the percentage of vital cells was assessed using a formula (vital cells/total cells×100%).

ELISA (Enzyme-linked immunosorbent assay)

Quantification of hormones (progesterone and 17β-estradiol) was performed after exposure of punicalagins by enzyme linked immunosorbent assay (ELISA). The principle of this colorimetric method is a series of competitive reactions between antigens (hormones) in the sample with horseradish enzyme-labelled antigen for binding to the limited number of antibody sites within a 96-well microplate (Grainer, Germany). ELISA assays (Dialab, Wiener Neudorf, Austria) were performed according to the manufacturer’s instructions. After 1 h incubation (37°C, 95 % air atmosphere, 5 % CO2) the bound/free separation was performed by a simple solid-phase washing. The enzyme substrate (H2O2) and the TMB-Substrate were added. After the appropriate time was elapsed for maximum colour development, the enzyme reaction was stopped and the absorbancies were
determined. Hormone concentration in the sample was calculated based on a series of calibrators. The colour intensity was inversely proportional to the hormone concentration in the sample. The absorbance was determined at a wavelength 450 nm using a microplate ELISA reader (Thermo Scientific Multiskan FC; Vantaa, Finland). The results were evaluated by One Way ANOVA. Intra- and inter-assay coefficients for progesterone were ≤5 % and ≤9.3 %. For 17β-estradiol intra- and inter-assay coefficients were ≤9 % and ≤10 %. Sensitivity for progesterone was 0.05 ng.ml⁻¹ and 8.68 pg.ml⁻¹ for 17β-estradiol.

RESULTS AND DISCUSSION

In our previous in vitro study punicalagins were described as a possible effector in the processes of ovarian steroidogenesis (Packová et al., 2015). Nagata et al. (2000) have shown that pomegranate juice is a potent inhibitor of CYP2C9 and CYP3A enzymes – these enzymes belong to cytochrome p450 group; and are responsible for cholesterol, steroid and lipid metabolism or synthesis. This study was focused on steroid hormones and the influence of punicalagins on the secretion of steroid hormones – progesterone and 17β-estradiol. Fig. 1 describes the secretion of progesterone as the first important hormone of steroid synthesis. Punicalagin had no significant (P≥0.05) impact on secretion of progesterone. Similarly, Ming et al. (2014) described a significantly decreased progesterone in the samples (prostate cancer cell lines – 22RV1 and LNCaP) treated with pomegranate extracts. However in our previous study, punicalagin at 100 µg.ml⁻¹ increased the progesterone secretion by rabbit ovarian cells (Packová et al., 2015).

CONCLUSION

The research was focused on possible effects of punicalagins on porcine ovarian granulosa cells. Punicalagins or its derivatives (ellagic acid etc.), which were used in the present study might have no effect on steroidogenesis by porcine ovarian granulosa cells, but our previous studies with rabbit ovarian fragments have shown that punicalagin affects the secretion of steroid hormones (progesterone and 17β-estradiol). Further research is necessary for a complex conclusion. There are more questions related to the effect of punicalagins or compounds from pomegranate on regulation of ovarian cells.

REFERENCES


