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Effect of Tumor Necrosis Factor α on Steroidogenesis in Cultured Human Ovarian Granulosa Cells

Wpływ czynnika nekrotyzującego guza α na sterydogenezę w hodowanych komórkach ziarnistych jajnika kobiety

Tumor necrosis factor α (TNF-α, cachectin) discovered by Carswell et al. (3) is a peptide responsible for tumor necrosis in mice. Lipopolysaccharide stimulate TNF-α production by different cells including macrophages (3, 4). Macrophages are the main source of TNF in the ovary (2), however, granulosa cells (GC) are capable to produce this cytokine (8, 10). TNF concentration in follicular fluid (ff) increased from the early follicular phase and reached the highest values in periovulatory follicle (10). TNF-α receptors were found on porcine granulosa cells (9). The main aim of the study was to examine *in vitro* effect of TNF-α on estradiol production by human GC obtained from follicles in different stages of development in relation to follicular volume, number of the obtained cells and concentration of LH, FSH, PRL in follicular fluid.

MATERIAL AND METHODS

Follicular fluid containing granulosa cells was aspirated from the largest ovarian follicle during laparotomy performed for non-ovarian reasons. Follicular aspirate volume was measured and centrifuged at 1500 rpm for 10 min. Supernatant was frozen in −20°C for further hormonal estimation. The cell pellet after washing in medium (Eagle 1559) was dispersed in culture medium (Eagle 1559, 5% FCS, Ampicyllin 100 μg/ml, Streptomycin 50 μg/ml). The number of the cells was estimated using hemacytomete. Cells from each follicle were placed separately in culture bottles and cultured in 3 ml of medium at 37°C, in an atmosphere of 5% CO_2 and air. Supernatant was replaced by 5 ml of fresh medium after 6 hrs of preincubation. Except controls either 100 and 500 ng/ml TNF-α (kindly provided by Dr W. J. Stec, Zakład Chemii Bioorganicznej — Bioorganic Chemistry Dept. — CBMiM PAN, Łódź) or LPS (10 μg/ml, Sigma, USA) were added to the culture. Portions (0.85 ml each) of media were collected after 6, 18, 42 and 66 hrs of culture and frozen until estradiol determination. Concentrations of LH, FSH, PRL and estradiol in follicular fluid and media were determined by radioimmunological methods.

Wilcoxon test was performed for data comparison. Correlation between groups was estimated by Spearman test. Values were considered significant when $p \leqslant 0.05$.

RESULTS AND DISCUSSION

Results were summarised in Table 1. In our study TNF inhibited estradiol production by cultured granulosa cells in all experiments after 6 hrs ($p \leqslant 0.05$) — Fig. 1. Decrease in estradiol secretion in 6 hr culture loaded with cachectin (100 ng/ml) was higher when cells originated from follicle with higher concentration of FSH in ff ($rs = 1$, $p = 0.00$). Robby and Terranova (9) did not

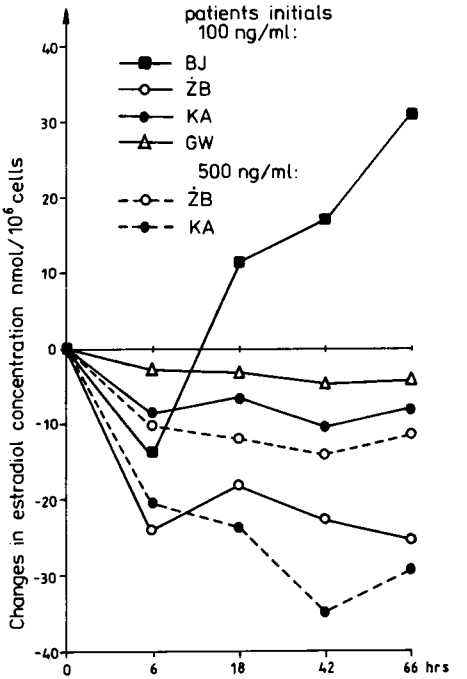

Fig. 1. Changes in estradiol concentration in media of cultures loaded with TNF

observe influence of TNF on estradiol secretion in 24 hrs rat GC culture. We confirmed results presented by Adashi et al. (1) and Emoto and Baird (5) that TNF inhibits FSH stimulated aromatization of androgens to estrogenes in cultured GC. According to Adashi group opinion (1) TNF in applied concentration did not influence GC proliferation, so we can draw the conclusion that the decrease in estradiol production was not induced by toxic effect of TNF. Cells washing exclude the possible influence of ffFSH on the results. Also no FSH in culture media before experiment was detected by RIA (sensitivity of the test 0.05 IU/L). The correlation between FSH concentration in follicular fluid

Table. 1. Concentration of hormones in follicular fluid (ff) and 17β estradiol accumulation in media after 6, 18, 42 and 66 hrs culture loaded with TNF-α (100 and 500 ng/ml) or LPS (10 μg/ml)

Patients initials		BJ	ŻB	KA	GW
ff volume (ml)		3.3	3.5	0.5	1.0
Cell number (× 1000)		130	150	650	100
FSH concentration in ff (IU/L)		1.6	2.4	1.3	0.9
LH concentration in ff (IU/L)		2.7	12.7	1.7	2.9
PRL concentration in ff (IU/L)		856	794	325	292
	hrs	Estradiol (nmol/10^5 cells) accumulation in culture media			
Control culture	6	64.3	54.9	68.9	15.0
	18	64.5	61.9	72.9	16.7
	42	58.9	64.6	74.8	16.5
	66	48.0	65.5	71.6	17.8
Medium + LPS	6	57.3			14.0
	18	62.5			14.1
	42	61.3			14.9
	66	50.9			17.7
Medium + TNF-α 100 ng/ml	6	50.0	31.1	60.3	12.2
	18	75.9	44.0	66.4	13.5
	42	77.9	42.0	64.5	11.9
	66	78.8	40.2	63.8	13.7
Medium + TNF-α 500 ng/ml	6		44.8	48.5	
	18		50.1	49.6	
	42		40.6	40.0	
	66		44.2	42.4	

and the extent of decrease in estradiol production could be explained exclusively by the dependence of granulosa cells metabolism *in vitro* on the condition in the follicle *in vivo*. It is in agreement with the previous observation (6, 7), that the influence of the follicular environment on granulosa in culture persists for at least 48 hrs.

Slight estradiol concentration differences in control culture and culture loading with lipopolysaccharides exclude effect of bacterial LPS contamination in recombinant cytokine.

No correlation was found between TNF dependent changes in estradiol production and cell number, volume and concentrations of LH, PRL in follicular fluid.

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STRESZCZENIE

Komórki ziarniste jajnika otrzymane po odwirowaniu płynu uzyskanego poprzez aspirację zawartości największego pęcherzyka gonady umieszczano w medium hodowlanym bez oraz z dodatkiem kachektyny (100 i 500 ng/ml) lub lipopolisacharydów (10 μg/ml). Po 6, 18, 42 i 66 godz. w medium z hodowli oznaczano metodą radioimmunologiczną zawartość estradiolu.

Kachektyna w stężeniu 100 i 500 ng/ml po 6 godz. hodowli obniżała uwalnianie estradiolu przez komórki ziarniste jajnika w odniesieniu do wartości w medium hodowli kontrolnej. Stwierdzono dodatnią korelację pomiędzy obniżeniem stężenia estradiolu w hodowli z kachektyną w stężeniu 100 ng/ml i koncentracją FSH w płynie pęcherzykowym, z którego pochodziły hodowane komórki. Lipopolisacharydy w stężeniu 10 μg/ml nie wpływały na uwalnianie estradiolu przez komórki ziarniste jajnika w hodowli.