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蒿甲醚对日本血吸虫葡萄糖摄取和糖元含量的影响

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关键词 日本血吸虫；葡萄糖；糖原；碳放射性同位素；蒿甲醚

目的：研究蒿甲醚(Art)对血吸虫摄入葡萄糖和糖原含量的影响. **方法**：用 Art 300 mg·kg^{-1} ig 治疗感染小鼠后 24－48 h 取虫，作体外培养，测定♀、♂虫糖原含量，对[U-^{14}C]葡萄糖的摄入及[U-^{14}C]葡萄糖掺入虫糖原的量. **结果**：感染小鼠用 Art 治疗后 24－48 h 取虫作体外培养 1－24 h，♂和♀虫的糖原含量分别减少 27 %－61 %和 39 %－78 %；6 组♂虫中，仅 3 组的葡萄糖摄入减少 23 %－35 %，而♀虫各组的均明显减少，减少率为 18 %－38 %. 除 2 组♂虫外，[U-^{14}C]葡萄糖掺入虫的糖原量无明显变化. **结论**：Art 引起血吸虫糖原的减少，可能与干扰糖酵解而不是与葡萄糖的摄入有关.

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Effect of DL111-IT on progesterone biosynthesis and viability of rat luteal cells *in vitro*[1]

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KEY WORDS DL111-IT; corpus luteum; progesterone; pregnenolone; forskolin; cultured cells; cell survival; chorionic gonadotropins

AIM: To study the influence of DL111-IT on progesterone biosynthesis of cultured luteal cells (LC). METHODS: LC viability was assessed with trypan blue dye exclusion and progesterone concentration was measured with radioimmunoassay. RESULTS: DL111-IT decreased the viability of LC after 24-h incubation, its ED_{50} being 7.7 (95 % confidence limits: 7.1－8.5) mg·L^{-1}. DL111-IT inhibited basal secretion of progesterone in a concentration-dependent manner, and 3 mg·L^{-1} decreased progesterone concentration by 25 % *vs* control. DL111-IT 3 mg·L^{-1} also inhibited the stimulatory effect of forskolin (cAMP activator) 10 μmol·L^{-1} and pregnenolone [converted to progesterone by 3β-hydroxysteroid dehydrogenase-isomerase complex (3β-HSD)] 10 μmol·L^{-1} on progesterone production in cultured LC, and their inhibitory rates were 43 % and 155 %, respec-

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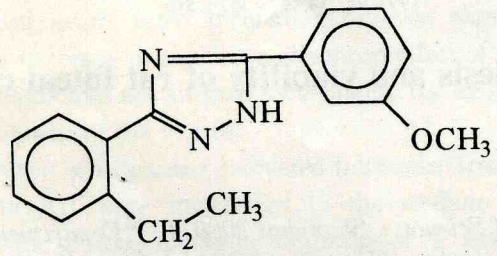
tively. At the same concentration, DL111-IT did not influence hCG-induced progesterone production. CONCLUSION: DL111-IT inhibited progesterone synthesis by suppressing the conversion of pregnenolone to progesterone (inactivating 3β-HSD) and suppressed the activity of cAMP. DL111-IT 6 −24 mg·L^{-1} decreased the viability of LC.

Corpus luteum is the primary site of progesterone biosynthesis during early stages of pregnancy. Progesterone is necessary to regulate a normal cycle, promote embryo implantation, and maintain normal pregnancy in females. The production of progesterone can be stimulated by hCG, pregnenolone (substrate of progesterone), and forskolin (activator of cAMP)[1].

DL111-IT, a nonhormonal compound[2], has a high contragestational activity in rodents, dogs, and primates. Cultured with decidual cells, it reduced quantity of progesterone receptors[3]. DL111-IT increased the efficacy of mefipristone on termination of early pregnancy of rats[3].

The present study intended to investigate the influence of DL111-IT on viability and progesterone production of cultured luteal cells.



3-(2-Ethyl phenyl)-5-(3-methoxy phenyl)-1H-1,2,4-triazole (DL111-IT)

MATERIALS AND METHODS

Drugs and reagents DL111-IT was manufactured by Zhejiang Xianju Pharmaceutical Co (Lot № 9306002). Collagenase (type II), forskolin, pregnenolone, and McCoy's 5A medium were purchased from Sigma Chemical Co. Progesterone radioimmunoassay kits were obtained from Research Center for RIA Reagents, Shanghai Institute of Biological Products.

Rats Sprague Dawley rats (clean, ♀; $n = 400$, 22 − 25 d, Shanghai Experimental Animal Center, Certificate № 005 conferred by Animal Management Committee, Chinese Academy of Sciences) were housed in an air-conditioned room and fed a standard rat chow with water *ad lib*. Each rat was injected sc pregnant mare serum gonadotropin (PMSG) 65 IU, 65 h later sc human chorionic gonadotropin (hCG) 35 IU, and was killed by cervical dislocation on d 5 after sc hCG for collection of luteal cell[4].

Luteal cell culture[5] Both ovaries were excised from pseudopregnant rats. Corpora lutea were dissected out under a microscope and seeded at a density of $(2-3)\times10^5$ cells/well in 0.5 mL McCoy's 5A medium supplemented with 10 % BSA, benzylpenicillin potassium 25 IU·L^{-1}, streptomycin 0.1 g·L^{-1} for 24 h. Pregnenolone 10 μmol·L^{-1}, forskolin 10 μmol·L^{-1}, or hCG 10 IU·L^{-1} were added with or without DL111-IT (1.5 − 24.0 mg·L^{-1}). Four wells were used in each dosage. Besides, 4 wells received media or vehicle (0.2 % EtOH) as control group. Cells were cultured at 37 ℃ in 5 % CO_2 for 24 h. Cell viability was assessed with typan blue dye exclusion. At the end of each treatment, the medium was stored at −20 ℃ for progesterone determination.

Progesterone determination The progesterone contents were determined with radioimmunoassay[3]. The intra- and inter-assay coefficients of variation were 3 % and 10 %, respectively. All standards and unknowns were performed in duplicate.

Statistical analysis Results were expressed as $\bar{x} \pm s$ and compared by t test. ED_{50} of cultured cells was calculated[6]. Each experiment was repeated 5 times.

RESULTS

Viability of rat luteal cells After rat luteal cells were exposed to 0.2 % (vol/vol) EtOH for 24 h, the viability was unchanged *vs* media. With the concentration of DL111-IT increased, the cell viability dropped *vs* control. ED_{50} (95 % confidence limits) was 7.7 (7.1 − 8.5) mg·L^{-1}. ED_5 and ED_{95} were 3.1 and 19.5 mg·L^{-1}, respectively. DL111-IT 1.5 and 3.0 mg·L^{-1} showed no effect on the cell viability.

Progesterone production

Basal secretion The progesterone production of rat luteal cells was inhibited in a concentration-dependent manner by DL111-IT (1.5 − 24 mg·L^{-1}). DL111-IT 3 mg·L^{-1} decreased ($P < 0.05$) progesterone production. Progesterone production and viability of LC decreased ($P < 0.01$) when exposed to DL111-IT 6 − 24 mg·L^{-1}. After DL111-IT 24 mg·L^{-1} was added, only 4 % LC was viable, a certain amount of progesterone was

still detected (Tab 1). The presence of 0.2 % EtOH in the medium showed no effect on progesterone production.

Tab 1. Progesterone production and number of viable luteal cells in culture with DL111-IT for 24 h. $n=5$ samples (each sample = 4 wells), $\bar{x} \pm s$. $^{a}P>0.05$, $^{b}P<0.05$, $^{c}P<0.01$ *vs* 0 mg·L^{-1}.

DL111-IT, mg·L^{-1}	Progesterone, ng/10^6 cells	10^{-3} × Viable cells
0	182 ± 37	241 ± 28
1.5	154 ± 21^a	245 ± 41^a
3.0	138 ± 18^b	237 ± 47^a
6.0	114 ± 12^b	179 ± 36^b
12.0	86 ± 10^c	17 ± 7^b
24.0	95 ± 12^c	1 ± 1^c

hCG-induced progesterone secretion

Progesterone secretion of rat luteal cells stimulated by hCG (10 IU·L^{-1}) was increased ($P<0.01$) over the basal level. DL111-IT 1.5 and 3 mg·L^{-1} did not inhibit hCG-stimulated progesterone secretion ($P>0.05$), but 6 mg·L^{-1} reduced both the viability of cells and hCG-stimulated progesterone production (Tab 2).

Conversion of pregnenolone to progesterone

Addition of pregnenolone 10 μmol·L^{-1} increased ($P<0.01$) progesterone secretion by luteal cell, but DL111-IT 3 mg·L^{-1} inhibited the conversion of exogenous pregnenolone to progesterone. At low dose (1.5 mg·L^{-1}), the inhibition of pregnenolone conversion was very slight ($P>0.05$) (Tab 2).

Forskolin-stimulated progesterone production

Forskolin 10 μmol·L^{-1} increased ($P<0.01$) the production of progesterone by luteal cells. Treated with DL111-IT 3 mg·L^{-1}, progesterone production by forskolin was inhibited remarkably ($P<0.01$). Even at 1.5 mg·L^{-1}, DL111-IT decreased ($P<0.05$) the production of progesterone (Tab 2).

Tab 2. Progesterone production of luteal cells cultured with hCG, pregnenolone, or forskolin, alone or combined with DL111-IT for 24 h. $n=5$ samples (each sample = 4 wells), $\bar{x} \pm s$. $^{a}P>0.05$, $^{c}P<0.01$ *vs* 0 mg·L^{-1}; $^{d}P>0.05$, $^{e}P<0.05$ *vs* hCG 10 IU·L^{-1}; $^{g}P>0.05$, $^{h}P<0.05$, $^{i}P<0.01$ *vs* pregnenolone 10 μmol·L^{-1}; $^{j}P>0.05$, $^{k}P<0.05$, $^{l}P<0.01$ *vs* forskolin 10 μmol·L^{-1}.

DL111-IT, mg·L^{-1}	Progesterone, ng/10^6 cells	10^{-3} × Viable cells
0	187 ± 43	252 ± 40
hCG 10 IU·L^{-1}	287 ± 54^c	261 ± 38^a
1.5 + hCG 10 IU·L^{-1}	284 ± 48^d	258 ± 50^d
3.0 + hCG 10 IU·L^{-1}	289 ± 46^d	243 ± 46^d
6.0 + hCG 10 IU·L^{-1}	210 ± 19^e	190 ± 37^e
0	215 ± 24	278 ± 59
Pregnenolone 10 μmol·L^{-1}	541 ± 34^c	283 ± 47^a
1.5 + Pregnenolone 10 μmol·L^{-1}	500 ± 28^g	274 ± 38^g
3.0 + Pregnenolone 10 μmol·L^{-1}	464 ± 38^h	269 ± 34^g
6.0 + Pregnenolone 10 μmol·L^{-1}	297 ± 36^i	210 ± 32^h
0	186 ± 46	238 ± 50
Forskolin 10 μmol·L^{-1}	412 ± 37	231 ± 36^a
1.5 + Forskolin 10 μmol·L^{-1}	323 ± 46^k	241 ± 71^j
3.0 + Forskolin 10 μmol·L^{-1}	260 ± 50^l	223 ± 41^j
6.0 + Forskolin 10 μmol·L^{-1}	158 ± 42^l	173 ± 23^j

DISCUSSION

In the present study, we investigated the ability of DL111-IT to influence the progesterone production and the viability of the luteal cells. With the concentrations of DL111-IT increased, viable cell number and progesterone production decreased. At concentration 3 mg · L^{-1}, progesterone production decreased dramatically ($P<0.05$), but viability of LC changed insignificantly ($P>0.05$). This result pointed out that inhibitory action of DL111-IT on progesterone production was not due to the cell death. Under tested with 12 and 24 mg·L^{-1} of DL111-IT, LC were damaged seriously, but there were still a certain amount of progesterone in the media. A lot of LC disrupted, and the progesterone inside these cells was released into the media. So the decrease of progesterone production was not as serious as cell damage.

Pregnenolone was finally converted to progesterone by 3β-hydroxysteroid dehydrogenase-isomerase complex (3β-HSD) in luteal cells[7], which played a key role in the synthesis of progesterone. Addition of pregnenolone, as a substrate for 3β-HSD, greatly increased progesterone secretion. DL111-IT 3 mg · L^{-1} significantly inhibited this substrate-enhanced conversion. These results suggested that DL111-IT might inhibit the activity of 3β-HSD.

Both forskolin and hCG could induce

intracellular cAMP formation. cAMP stimulated progesterone production in cultured rat luteal cell through different pathways[8]. hCG increased intracellular cAMP level by receptor-mediated mechanism. Our data indicated that DL111-IT, only at higher dose which dramatically decreased LC, could inhibit hCG-induced progesterone production. This inhibitory action devoted mainly to the death of luteal cells. Forskolin increased intracellular cAMP level by nonreceptor-mediated mechanisms. DL111-IT 1.5 mg · L^{-1}, half of ED_5, inhibited forskolin-promoted progesterone production. This demonstrated that DL111-IT might prevent cAMP formation by non-receptor-mediated pathway. It was believed that during progesterone synthesis in luteal cells, the availability of intracellular cholesterol and the activates of steroidogenic enzymes are mediated by hormones and cAMP[1]. So, it was suggested that, DL111-IT affected the steroidogenesis at a site subsequent to cAMP formation in cultured luteal cells, or, in other words, affected the activity of cAMP.

In summary, the results suggested that DL111-IT inhibited basal progesterone production at low concentrations by suppressing the conversion of prognenolone to progesterone and the activity of cAMP. It also decreased the viability of rat luteal cells *in vitro*.

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抗孕唑对离体黄体细胞黄体酮生物合成和生存能力的影响[1]

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关键词 抗孕唑; 黄体; 黄体酮; 孕烯醇酮; 弗司扣林; 培养的细胞; 细胞存活; 绒毛膜促性腺激素类

目的: 研究抗孕唑(DL111-IT)对离体大鼠黄体细胞孕酮合成和存活率的影响. **方法**: 体外培养大鼠黄体细胞, 用细胞计数和放射免疫法. **结果**: DL111-IT 使黄体细胞存活率随剂量上升而下降, ED_{50}为 7.7 (7.1 – 8.5) mg·L^{-1}. DL111-IT 3 mg·L^{-1}使黄体细胞基础黄体酮分泌下降 25 %, 并可显著抑制弗司扣林(cAMP 激动剂) 10 μmol·L^{-1}和孕烯醇酮(可被 3β-HSD 催化成黄体酮) 10 μmol·L^{-1}的促孕酮分泌作用, 抑制率分别为43 %和 155 %, 但不抑制 hCG 10 IU·L^{-1}的促黄体酮分泌作用. **结论**: DL111-IT 抗早孕作用是在 cAMP 和 3β-HSD 水平上影响基础黄体酮分泌功能, 并于高剂量降低黄体细胞成活率.