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大鼠在胚泡着床前应用阿司匹林与醋氨酚对胚泡及胎仔发育的影响[1]

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Effects of preimplantation treatment with aspirin and acetaminophen on blastocyst and fetus in rats

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ABSTRACT Pregnant rats were treated with ig aspirin (Asp) and acetaminophen (Ace) on d 3 of pregnancy (positive vaginal smear = d 0). Blastocysts were collected on d 4 and evaluated for gross morphology, cell number, micronucleus, and mitotic index. Some rats were killed on d 20 and fetuses were examined for teratogenic effects. On d 4 a reduction of cell number per blastocyst was found in the rats treated with Asp 0.5, 1 $g \cdot kg^{-1}$, and Ace 1 $g \cdot kg^{-1}$, while the mitotic index, frequency of micronuclei, and frequency of blastocysts with morphological alterations were increased. The frequency of micronuclei was increased in rats exposed to Ace 0.25 and 0.5 $g \cdot kg^{-1}$. On d 20 major malformation and embryotoxicity were seen in Asp 0.5, 1, and Ace 1 $g \cdot kg^{-1}$ groups.

KEY WORDS aspirin; acetaminophen; blastocyst; micronucleus tests; teratogens; fetus

摘要 大鼠受孕 d 3时 ig 阿司匹林(Asp)或醋氨酚(Ace), d 4收集胚泡, 观察其形态, 细胞数, 微核及分裂相细胞. 结果Asp 0.5, 1 $g \cdot kg^{-1}$及Ace 1 $g \cdot kg^{-1}$使胚泡细胞数减少, 形态异常率, 微核率及分裂指数增高. Ace 0.25与0.5 $g \cdot kg^{-1}$呈胚泡微核诱导作用. 两药对着床后胚胎有胚胎毒与致畸作用.

关键词 阿司匹林; 醋氨酚; 胚泡; 微核试验; 致畸胎物; 胎儿

药物对着床前胚胎作用一般呈"全或无"模式[1], 但并不全然[2-4]. 阿司匹林(aspirin, Asp)在啮齿类动物器官形成期给药具致畸作

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用[5]，小鼠怀孕前或妊娠期给醋氨酚(acetaminophen，Ace)可使胎仔大脑，垂体及体重减轻[6]. 本文研究妊娠大鼠胚泡着床前ig Asp及Ace，对胚泡及胎仔发育的影响，探索药源性胚泡异常与胎仔发育的关系.

MATERIALS AND METHODS

Asp为山东新华制药厂产品；Ace为苏州制药厂生产；环磷酰胺(cyclophosphamide，Cyc)为Sigma产品. Sprague-Dawley大鼠，♀，体重207±*s* 18 g，♂，体重289±*s* 25 g，浙江医科大学实验动物中心提供.

大鼠♀：♂ (4:1)于18:00合笼，翌晨8:00♀鼠作阴道涂片，查到精子当天为妊娠d 0，分笼饲养，分别以每组10—14鼠，于妊娠d 0，1，2及d 3单次ig Asp 0.5 g·kg^{-1}观察其时效关系；或以每组9—14鼠，于妊娠d 3分别ig Asp和Ace 0.25，0.5，1 g·kg^{-1}，作量效观察(两药1 g·kg^{-1}分别相当于大鼠LD_{50}的1/2及1/3). 药物皆以0.5%羧甲基纤维素钠(CMC)配制悬液. 另设ig 0.5% CMC 5 ml·kg^{-1}作对照，ip Cyc 40 mg·kg^{-1}作阳性对照.

上述各组孕鼠于妊娠d 4 (13:00—16:00)处死，取子宫，以0.85% NaCl溶液冲出胚泡，收集于微型表面皿中，低倍显微镜(×40)下观察胚泡形态，记录胚泡形态异常率(Frequency of blastocysts with morphological alterations，FBMA)，再以直径5—10 μm玻管将胚泡转移至0.7%枸橼酸钠溶液中，低渗处理10 min，再将胚泡转移在玻片上，加数滴乙醇:冰醋酸(3:1，vol/vol)混合液固定，干燥后用Giemsa (pH 7.4)染色，显微镜(×1000)下记录胚泡细胞数(cell number per blastocyst，CNPB)，微核率(Frequency of micronuclei，FM)，具微核胚泡率(Frequency of blastocysts with micronuclei，FBM)与分裂指数(mitotic index，MI)[7]. 另各组以12—17只孕鼠于妊娠d 3给药，妊娠d 20处死，按常规[8,9]检查畸胎.

CNPB用*t*检验，FBMA，FM，FBM及MI用χ^2检验，作组间显著性检验.

RESULTS

Asp和Ace对着床前胚泡的影响 大鼠妊娠后d 0—3各单次ig Asp 0.5 g·kg^{-1}，妊娠d 3给药组CNPB较对照组减少($P<0.01$)，FBMA、FM与MI较对照组升高($P<0.05$，<0.01)；其余时间给药组、除MI (3.0%，3.3%及3.2%)较对照组升高($P<0.05$)外，CNPB，FBMA，FM及FBM均无明显差别($P>0.05$). 妊娠d 3时ig Asp 0.25 g·kg^{-1}，对胚泡各项观察指标，与对照组相比，无明显影响($P>0.05$)；其他各剂量组的CNPB减少($P<0.01$)，FBMA，FM及MI均升高($P<0.05$，<0.01) (Tab 1).

大鼠妊娠d 3时ig Ace 0.25与0.5 g·kg^{-1}，

Tab 1. Effects of ig aspirin and acetaminophen on d 3 of pregnancy on cell number per blastocysts (CNPB), frequency of blastocysts with morphological alterations (FBMA), frequency of micronuclei (FM), frequency of blastocysts with micronuclei (FBM), and mitotic index (MI) in rats. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

Group	Dose g·kg^{-1}	*n*	Blastocysts[1] recovered	used	CNPB ($\bar{x}$±*s*)	FBMA/ %	FM/ ‰	FBM/ %	MI/ %
Control		20	180	168	42±5	7.5	4.2	11.9	2.4
Cyc	0.04	12	107	98	33±7***	23.1***	31.9***	49.0***	3.8***
Asp	0.25	10	90	80	38±5*	13.2*	6.9*	13.8*	2.7*
	0.5	14	123	113	36±6***	18.2**	8.1***	15.0*	3.9***
	1	13	117	109	30±7***	34.1***	9.8***	13.8*	3.4***
Ace	0.25	9	83	63	40±8*	8.5*	15.9***	33.3***	2.7*
	0.5	10	79	64	40±8*	6.9*	14.8***	35.9***	2.3*
	1	10	81	75	34±4***	20.6**	19.0***	37.3***	3.2**

[1] Differences between recovered and used blastocysts were due to losses during manipulation.

Tab 2.　Maternal treatment with aspirin and acetaminophen on d 3 of gestation in rats：effects on dams and litters.　$\bar{x}\pm s$.　$^{*}P>0.05$，$^{**}P<0.05$，$^{***}P<0.01$ *vs* control.

	Control	Acetaminophen 1 g·kg^{-1}	Aspirin/g·kg^{-1} 0.25	0.5	1
Pregnant/mated dams	14/15	12/12	11/12	13/14	13/17
Maternal (d 0—20) weight gain (g)					
with litter	92±23	84±27*	89±13*	73±32*	68±25**
without litter	29±16	28±19*	31±14*	26±25*	34±14*
Fetal weight (g)	3.7±0.5	3.3±0.7*	3.6±0.3*	3.0±0.5***	3.0±0.5***
Preimplantation loss (%)	10.9	10.5*	9.6*	13.9*	31.6***
Postimplantation loss (%)	5.1	20.7***	12.2**	13.3**	27.9***
Fetuses examined	148	115	108	124	75
Fetuses with malformations	0	4*	1*	7**	9**
Dams with malformed fetuses	0	4**	1*	6***	5***
Fetuses wtih anomalies	26	41***	35***	56***	49***
Dams with anomalous fetuses	10	9*	9*	13**	12*

对 CNPB 和 FBMA 与对照组相比无明显影响，1 g·kg^{-1}使 CNPB 减少($P<0.01$)，FBMA 和 MI 升高($P<0.01$，<0.05)，Ace 各剂量组均使 FM 和 FBM 升高($P<0.01$)．阳性对照 Cyc 充40 mg·kg^{-1}使胚泡各项观察指标均有改变($P<0.01$) (Tab 1).

大鼠妊娠 d 3 ig Asp 或 Ace 对胎仔的影响　孕鼠于妊娠 d 20剖杀，Asp 1 g·kg^{-1}使着床前胚胎损失率较对照组增加($P<0.01$)，其余各剂量组及 Ace 1 g·kg^{-1}与对照组相比无明显差异($P>0.05$)．Asp 各剂量组和 Ace 1 g·kg^{-1}对着床后的胚胎损失率均较对照组增加($P<0.05$，<0.01)．Asp 0.5，1 g·kg^{-1}使畸胎数增加($P<0.05$)，且该两剂量及 Ace 1 g·kg^{-1}均使带畸胎的母鼠数及异常的胎仔数增多($P<0.01$，<0.05) (Tab 2).

胎仔外观、内脏及骨骼检查　Asp 0.5 g·kg^{-1}组有肾萎缩胎仔1只，睾丸缺失胎仔2只，脑积水和胸骨缺失胎仔各1只；Asp 1 g·kg^{-1}组脑积水胎仔4只，胸骨缺失胎仔1只．Ace 1 g·kg^{-1}组腹裂胎仔1只，主动脉弓异位胎仔1只，枕骨和胸骨缺失胎仔各1只．有异常的胎仔主要表现在骨骼方面，其中属颅骨异常的发生率，Asp 0.25，0.5，1 g·kg^{-1}和 Ace 1 g·kg^{-1}组分别占32.7%，30.8%，48.8%和26.2%，与对照组3.9%比较均显著增加($P<0.01$)；Asp 0.5与1 g·kg^{-1}胎仔胸骨异常率分别为58.5%和75.6%，均高于对照组(26.0%) ($P<0.01$)；Asp 1 g·kg^{-1}组，盆骨骨化不全(14.6%)和皮下出血的胎仔(6.7%)均显著增加($P<0.01$)；其它尚有胎仔舌骨体缺失和波状肋等.

Asp 1 g·kg^{-1}组母鼠体重在给药后2 d 内较对照组有减轻($P<0.01$)，但在孕后期及临产前与对照组比较，均无显著差异($P>0.05$).

DISCUSSION

有关检测药源性胚泡异常的研究，国内尚未见报道．本研究严格规定了动物交配，给药和取胚泡过程时间，以减少对 CNPB 等结果的影响．并设 Cyc 为阳性对照，结果与国外报道[10]基本一致．实验表明，孕鼠妊娠 d 3给 Asp 和 Ace 均可明显影响大鼠着床前胚胎发育．Asp 主要使 CNPB 减少，MI 升高，提示 CNPB 减少系细胞增殖受抑所致．两药均可显著地诱导胚泡产生微核，但 Asp 在导致 FM 提高时，并不影响 FBM；而 Ace 则能显著提高 FM 和 FBM，提示 Ace 对着床前胚胎具较广泛

的遗传毒性作用. Ace 对 DNA 的损伤主要在细胞毒水平发生[11]. 本研究表明 Ace 对 CNPB 无显著影响时，已有染色体损伤作用.

Giavini 等[7]曾报道，药源性着床前胚泡异常对胎仔发育的影响并不呈"全或无"模式，本文结果与此相符. 本实验还发现，畸胎率显著提高的剂量组均相应出现 CNPB 减少，FBMA，FM 及 MI 升高，提示药源性着床前胚泡异常与着床后畸胎发生可能有内在联系.

Asp 0.25 $g \cdot kg^{-1}$未见对胚泡有明显影响，而使着床后胚胎毒和胎仔异常率增加，因此，就 Asp 而言. 在细胞学水平观察药物对胚泡的影响尚不够深入. 本实验还不能排除因药物引起母体毒性，而导致着床前，后胚胎丢失及畸胎发生的可能性.

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可乐定对大鼠动情期、雌二醇、促性腺激素、格雷夫氏卵泡和黄体的影响[1]

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R 965.2

Effects of clonidine on estrus, estradiol, gonadotropin, Graafian follicle, and corpus luteum in rats

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ABSTRACT Effects of clonidine (Clo) on ♀ reproductive system were studied in rats. Blood FSH, LH, progesterone, testosterone, and estradiol were mea-