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Mutagenicity tests on epristeride *in vitro* and *in vivo*

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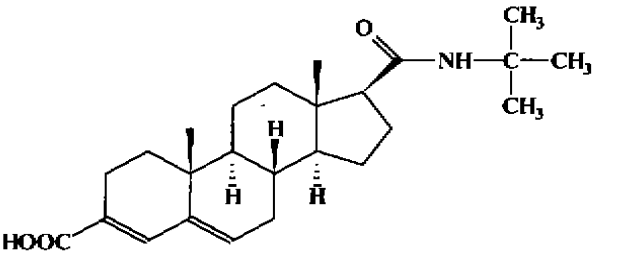
KEY WORDS epristeride; mutagenicity tests; chromosome aberrations; micronucleus tests; sperm count

AIM: To evaluate the genetic effects of epristeride (Epr), a new prospective drug for treating benign prostatic hyperplasia. **METHODS:** 1) Assaying reverse mutation in histidine nutritional deficiency strain of *Salmonella typhimurium* 2) detecting chromosome aberrations in Chinese hamster lung cells (CHL); 3) micronucleus assays of mouse bone marrow; 4) counting sperm shape abnormalities 35 d after first ig Epr. **RESULTS:** 1) The reverse mutation happened at almost the same rate of the negative control. Epr did not induce bacterial mutation. 2) *In vitro*, the rates of aberration were all below 3 %, thus Epr did not induce chromosome damage in CHL. 3) Micronucleated polychromatic erythroblasts (PCE) were not apparently more than those of sovent control, Epr did not induce the formation of micronuclei in PCE. 4) With Epr 818, 682, and 341 mg · kg^{-1}, the head abnormalities of sperms were 5.3 % ± 2.7 %, 5.3 % ± 1.9 %, and 5.2 % ± 1.2 %, respectively. **CONCLUSION:** No genetic toxicity of Epr was detected.

Benign prostatic hyperplasia (BPH) is a distressful condition in a majority of aging men. It results in a variety of symptoms related to difficulties of urination by the enlarged prostate gland[1]. Epristeride (Epr, 17β-*tert*-butylcarbamoyl-androsta-3,5-diene-3-carboxylic acid, SK&F 105637), another steroidal 5α-reductase inhibitor developed after finasteride (MK-906 or Proscar), is undergoing clinical evaluation and being introduced into the market for the therapy of BPH[2]. 5α-reductase is an intracellular enzyme which converts testosterone to dihydrotestosterone (DHT) and plays an important role in maintaining the balance between cell proliferation and death in prostate[3]. So it becomes necessory to test potential effects of Epr. This study was to investigate the mutagenic effects of Epr to provide safety data for its clinical application.



Epristeride

MATERIALS AND METHODS

Reagents and drugs Epr (purity >99.6 %) was synthesized by Prof LIAO Qin-Jiang (China Pharmaceutical University); mitomycin C (Mit, Kyowa Hakko Kogyo Co); cyclophosphamide (Shanghai Twelfth Pharmaceutical Factory). Other chemicals were of AR.

Ames test Strains of *Salmonella typhimurium* (TA97, TA98, TA100, and TA102) were kindly presented by Prof B N Ames (Department of Biochemistry, UCSF, USA). Their genotypes were checked here before tests. Chinese hamster lung (CHL) cells were from Shanghai Institute of Cell Biology. Rat-liver S9 was prepared via Aroclor 1254 induction[4]. The plate incorporation test was used for deficient metabolic system experiment and preincubation was used for + S9 system[5].

Detection of chromosome aberrations CHL cells were used. The IC_{50} of Epr was selected as the highest dose level. Epr 0.1 mL was added at a final concentration of 240, 120, or 60 mg·L^{-1} (each for 3 bottles). In negative group, 0.1 mL of physiologic saline took instead of Epr samples. In positive group, Mit took the place of Epr. Another set of cultures were added 0.5 mL of S9-mix as metabolic parallels; cyclophosphamide (Cyc) served as positive

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control[6]. CHL cells were arrested by Colecemid at the metaphase stage of mitosis. The conventional classification system[7] was adopted.

Micronucleus (MN) assay ICR mice (♂ 25, ♀ 25, 18 – 22 g), purchased from SIPPR/BK Laboratory Animals Ltd (SPF level), were treated once with Epr 175 mg·kg^{-1} ig. To find optimal time of detecting MN, multiple samplings were finished 12, 18, 24, 36, 48, and 72 h later. However, no positive results emerged in all samplings. Therefore sampling was detected for MN 24 h after treatment with Epr 350, 175, and 88 mg·kg^{-1} [5]. The negative and positive control groups were given 0.5 % carboxymethylcellulose (CMC, containing 10 % propylene glycol) and Cyc, respectively.

Sperm morphology test Kunming mice (♂, 18 – 22 g), were from Shanghai Experimental Animal Center, Chinese Academy of Sciences (Grade Ⅱ, Certificate No 005). The single-exposure LD_{50} (CL_{95} of Epr for male mice was determined as 682 (601 – 773) mg·kg^{-1}. Through dose-find tests, 3 levels of Epr were set in the test: 818, 682, and 341 mg·kg^{-1}. Mice were given Epr ig for 5 d. With the same volume, CMC was used as solvent vehicles and negative control. Mit was ip injected as positive control. All mice were killed by cervical dislocation 35 d after the first dose. Epididymides were minced in PBS, dispersed, and filtered with double-layer gauze to exclude large tissue fragments. Smears were prepared on slides and stained with 0.05 % eosin Y (aqueous). Sperm smears were examined under light microscope at 400 magnification with a blue filter. In each dose group, by counting 200 sperms per mouse and at least 1000 sperms totally were assessed for morphological head abnormalities[8].

RESULTS

In Ames test, Epr little affected the growth of all bacteria even up to 5 mg per plate and showed good background at all dose levels. There was almost no difference between negative control and Epr-treated groups as regards the number of revertant colonies per plate whether or not S9-mix was added (Tab 1, 2).

Tab 1. Effect of epristerider on reversion mutation of *S typhimurium* in Ames test (without S9). ① 9-aminoacridine 50 μg/plate; ② *p*-nitroquinoline 200 μg/plate; ③ methyl methanesulfonate 1 μL/plate; ④ mitomycin 5 μg/plate. $n = 6$ plates. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* negative control.

Dose μg/plate	Number of revertant colonies			
	TA97	TA98	TA100	TA102
Negative control	115 ± 4	22 ± 4	158 ± 32	283 ± 13
Epr 5	111 ± 19^a	17 ± 4^a	123 ± 12^a	194 ± 45^a
50	105 ± 12^a	21 ± 5^a	134 ± 34^a	253 ± 8^a
500	116 ± 5^a	20 ± 2^a	106 ± 4^a	249 ± 11^a
2 000	102 ± 13^a	19 ± 2^a	118 ± 2^a	206 ± 10^a
5 000	117 ± 1^a	21 ± 1^a	121 ± 8^a	211 ± 8^a
Positive control	① 3 065 ± 103^c	② 1 123 ± 89^c	③ 3 508 ± 167^c	④ 3 564 ± 189^c

Tab 2. Effect of Epr on *S typhimurium* in Ames test (with S9). ① 2-aminofluorene 10 μg/plate; ② 1,8-dihydroxyanthraquinone 50 μg/plate. $n = 6$ plates. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* negative control.

Dose μg/plate	Number of revertant colonies			
	TA97	TA98	TA100	TA102
Negative control	198 ± 10	37 ± 6	191 ± 33	235 ± 12
Epr 5	159 ± 8^a	31 ± 2^a	109 ± 22^a	243 ± 4^a
50	195 ± 4^a	36 ± 7^a	129 ± 23^a	266 ± 10^a
500	157 ± 17^a	27 ± 3^a	134 ± 12^a	240 ± 10^a
2 000	159 ± 11^a	25 ± 2^a	114 ± 6^a	214 ± 13^a
5 000	161 ± 8^a	20 ± 2^a	111 ± 8^a	193 ± 5^a
Positive control	① 2 576 ± 151^c	① 3 104 ± 211^c	① 1 523 ± 189^c	② 1 868 ± 98^c

After CHL cells were arrested by Colecemid at the metaphase stage of mitosis, the chromosome aberrations was detected by scoring the slides. Epr even at high concentration, eg, 240 $mg \cdot L^{-1}$, induced chromosome aberration at low frequency similar to negative control no matter whether or not S9 metabolic system was present (Tab 3).

Tab 3. Chromosome aberration inducted by Epr in CHL (without S9). Types of aberration: B: chromatid or chromosomal breaks; P: polyploid; T: translocation; E: exchange. Score: (−) negative <5 %; (±) suspicious 5 %−9 %; (+) positive 10 %−19 %; (++) 20 %−49 %; (+++) >50 %.

Group	Dose/ $mg \cdot L^{-1}$	Exposure time/h	Type of aberration B	T	P	E	Rate of aberration	Score
Me_2SO	0.1 mL	24			3		3 %	−
		48			3		3 %	−
Mit	0.2	24	24	10		5	32 %	++
	0.2	48	26	26	2	4	49 %	++
Cyc	20	24	45	46	2	6	63 %	+++
Epr	240	24			3		3 %	−
		48			3		3 %	−
	120	24			3		3 %	−
		48			2		2 %	−
	60	24			2		2 %	−
		48			2		2 %	−

In MN assays, the formation of micronucleated polychromatic erythroblast was induced below 0.3 %. There was no significant difference between Epr-treated group and negative group (Tab 4).

Tab 4. Effect of Epr on formation of micronucleated polychromatic erythroblasts (PCE). $n = 10$ mice, $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* control.

Group	Dose/ $mg \cdot kg^{-1}$	Total PCE	Micronucleated PCE n	/%	PCE/NCE
Control	0.2 mL	10 000	20	0.18 ± 0.13	1.49 ± 0.27
Epr	88	10 000	18	0.17 ± 0.14[a]	1.34 ± 0.19[a]
	175	10 000	26	0.25 ± 0.9[a]	1.31 ± 0.35[a]
	350	10 000	22	0.21 ± 0.10[a]	1.51 ± 0.24[a]
Cyc	60	10 000	324	4.0 ± 1.6[c]	1.24 ± 0.32[a]

During sperm morphology test, it was in accordance with the protocols that some mice died in each Epr-treated group. By counting the number of sperm head abnormalities, no significant difference was found between Epr-treated group and negative group although there was a slightly increase in the former (Tab 5).

Tab 5. Effects of Epr on sperm head morphology in mice. $^aP > 0.05$ *vs* CMC control. $^cP < 0.01$ *vs* Mit.

Group	Dose/ $mg \cdot kg^{-1}$	Treated mice	Survived mice	Head abnormalities/ %
CMC-Control	20 $mL \cdot kg^{-1}$	10	10	4.5 ± 0.8
Epr	818	15	5	5.3 ± 2.7[ad]
	682	15	6	5.3 ± 1.9[ad]
	341	10	7	5.2 ± 1.2[ad]
Mit	1.5	10	10	16.7 ± 3.3

DISCUSSION

Epr is a potent and selective uncompetitive inhibitor of the prostatic steroid 5α-reductase isoform 2[9]. It is known that 5α-reductase distributes widely in the body; therefore, Epr may bring some unexpected effects in many tissues. It is necessary to assess the safety from various aspects. Before Epr was adopted clinically, we devised and carried out the experiment to assess genetic effects of Epr at different levels, ie, gene mutation, chromosome aberration, and *in vivo* system as showed above. Both bacterial mutation assays and assays for detecting chemically induced chromosome aberration in cultured mammalian cells have revealed that Epr did not engender gene mutations or chromosomal mutations *in vitro*, either directly incurring or through metabolic activation of S9. However, provided the accretion of sperm body abnomalities, it was suggested that Epr could affect the quality of sperms. Considering bone marrow and spermatogenic tissue are rich source of mitotic cells, bone marrow assay and sperm morphology test therefore provide dependable assessment of chromosomal damage in living organisms as a suppliment to *in vitro* tests. As a result, Epr did not exhibit any apparent harmful effects.

Taking account of all these results, it is plain that Epr does not bring forward mutagenic effects either *in vitro* or *in vivo*.

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依立雄胺体内外致突变实验

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关键词 依立雄胺；致突变试验；染色体畸变；微核试验；精子计数

目的：评价治疗良性前列腺肥大的新药依立雄胺（Epr）的遗传影响. **方法**：1）鼠沙门氏菌体外回复突变试验测试能否诱发基因突变；2）CHL 细胞染色体的损伤和畸变实验；3）ICR 小鼠一次 ig Epr 后测试是否导致骨髓嗜多染红细胞染色体的损伤；4）昆明种小鼠连续 ig Epr 5 d，30 d 后统计精子头部异常情况. **结果**：1）Epr 不诱导细菌回复突变. 2）CHL 细胞染色体畸变低于 3 % 不造成细胞染色体损伤. 3）Epr 不诱导小鼠嗜多染红细胞微核的形成. 4）Epr 高、中、低剂量组引起的头部畸形率分别为 5.3 % ± 2.7 %，5.3 % ± 1.9 %，5.2 % ± 1.2 %，与对照组相比不引起显著的精子头部异常. **结论**：Epr 在体内外实验中没有表现出遗传毒性.

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多塞平对离体兔基底动脉环和隐动脉环的作用

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Effects of doxepin on isolated basilar and saphenous artery rings of rabbits

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KEY WORDS doxepin; norepinephrine; serotonin; arteries

AIM: To study the effects of doxepin (Dox) on cerebral artery. **METHODS**: The effects of Dox were observed using the isolated basilar and saphenous artery rings of rabbits. **RESULTS**: Dox inhibited the constriction of the basilar and saphenous artery rings evoked by KCl with IC_{50} 5.75 $\mu mol \cdot L^{-1}$ (95 % confidence limits were 2.3 – 14 $\mu mol \cdot L^{-1}$, $n = 8$) and 34.6 $\mu mol \cdot L^{-1}$ (95 % confidence limits were 3.8 – 316 $\mu mol \cdot L^{-1}$, $n = 8$), respectively. Dox also inhibited the constriction of the basilar and saphenous artery rings of the rabbits stimulated by 5-hydroxytryptamine (5-HT), IC_{50} were 6.3 $\mu mol \cdot L^{-1}$ (95 % confidence limits were 1.7 – 23.3 $\mu mol \cdot L^{-1}$, $n = 7$) and 8.0 $\mu mol \cdot L^{-1}$ (95 % confidence limits were 6.3 – 10.3 $\mu mol \cdot L^{-1}$, $n = 6$), respectively. In both samples (basilar and saphenous artery rings) $CaCl_2$ evoked, the

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