Effect of sodium artesunate on pregnancy in rats

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ABSTRACT **OBJECTIVE:** To study the effect of sodium artesunate (SA) on content of progesterone and decidua of pregnant rats and investigate the efficacy and mechanism of SA for termination of early pregnancy. **METHOD:** Serum content of progesterone was measured with RIA. The effect of SA on the overy, decidua and fetus of pregnant rats were studied using histochemistry techniques. Decidual cells were estimated using cell culture. **RESULTS:** SA 40 $mg \cdot kg^{-1} \cdot d^{1}$ given sc on d 6-10 of gestation significantly decreased the concentration of serum progesterone in early pregnant rats; decidual cells and fetus of treated groups were found to be degenerated at d 11. SA was shown to directly damage the decidual cells. Cultured human decidual cells were exposed to atesunate for 48 h, LC_{50} was found to be 25.18 ± 3.49 $ml \cdot L^{-1}$. **CONCLUSIONS:** SA had a feticide effect in rats and the damage on decidua and placenta may be the mechanisms of its contragestational action.

KEY WORDS sodium artesunate, progesterone, decidua, termination of pregnancy

青蒿琥酯钠对大鼠抗孕作用的研究

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摘要　目的:研究青蒿琥酯钠(SA)对大鼠的抗孕作用以及其对妊娠及假孕大鼠血清孕酮含量和蜕膜组织的影响,以探讨其终止妊娠的机理。方法:采用放射免疫分析法(RIA)测定血清孕酮含量;HE 染色法观察 SA 对大鼠子宫内膜的影响;用人蜕膜细胞体外培养方法观察 SA 对蜕膜细胞的直接作用。结果:SA 40 $mg \cdot kg^{-1} \cdot 5d$ 可使妊娠和假孕大鼠血清孕酮水平显著下降($P < 0.05$),但假孕大鼠血清孕酮水平下降晚于妊娠大鼠;整体水平能损伤蜕膜组织乃至胎盘;对体外培养的人蜕膜细胞有直接杀伤作用,其 LC_{50} 为 25.18 ± 3.49 $ml \cdot L^{-1}$。结论:青蒿琥酯钠能使血清孕酮含量下降并损伤蜕膜和胎盘而使胚胎坏死、吸收而终止妊娠。

关键词　青蒿琥酯钠;孕酮;蜕膜;终止妊娠

Sodium artesunate (SA), a synthetic derivative of artemisinin firstly isolated from the Chinese herb Aremisia annua in China in 1970', is a water soluble antimalaria used clinically in treatment of acute cerebral and malignant malaria all over the world now[1]. The embrotoxicity and teratogenicity experiments in mice showed SA was embryotoxic. The dose of SA given in the middle and late stages of organogenesis to cause all fetuses absorbed in mice was very low[2]. It was also found that SA has efficacy in terminating pregnancy in hamsters[3].

The present study intended to test the effect of SA on termination of early pregnancy in rats and investigate the influence of SA on serum progesterone concentration and decidual after rats administered sc dose of terminating pregnancy to explore the mechanism of its action of causing embryo absorbed.

1 Meterials

1.1 Animals and materials Sprague-Dawley rats (♀, 230 ± 20 g; ♂, 320 ± 20 g) were provided by Zhejiang University Medical Institute Animal Center. Ethical Human decidual cells were obtained from the healthy pregnant woman (6-9 wk) by vacuum aspiration of decidua, and distributed by Shanghai Ruijing Hospital.

1.2 Drugs and Reagents Artesunate (Art) and 5 % $NaHCO_3$ injection were manufactured by Guangxi Guilin No.2 Pharmaceutical Factory and Art was dissolved by 0.3 % $NaHCO_3$ to be SA before used. Progesterone redioimmunoassay kits were obtained from Tianjin Depu/DPC biological and medical products Inc. FD (F_{12} + Duibecco's modified Eagls's medium) culture medium was from Sigma.

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2 Rethods

2.1 Contragestational test Pregnant rats were randomly divided into 6 groups on d 6 and sc SA or 0.3% $NaHCO_3$ (as a control) for 5 d. Rats were mated at 16:00 every day and the day when spermatozoa were found in the vaginal smear was taken as d 1 of pregnancy. Rats were decapitated on d 15 and the ED_{50} of terminating pregnancy was calculated.

2.2 Preparation of pseudopregnant rats Three ♀ rats were placed overnight with one ♂ rat which had been given ig 3-chloro-1,2-proganediol 5 $mg \cdot kg^{-1} \times 5$ d to be deprived of fertility ability before mating[4]. The day when spermatozoa were found in the vaginal smear was taken as d 1 of pseudopregnancy.

2.3 Progesterone determination The pregnant and pseudopregnant rats were randomly divided into two groups (10 rats per group) and treated with SA 40 $mg \cdot kg^{-1} \cdot d^{-1}$ or 0.3% $NaHCO_3$ for 1, 3, 5 d (from d 6 of pregnancy or pseudopregnancy onwards). Blood samples were collected on d 7, d9, dl1, d 13 of pregnancy or pseudopregnancy. Blood samples from rats which were found not to be pregnant were discarded. The progesterone contents were determined with radioimmunoassay. The intra- and inter-assay coefficients of variation were 3% and 10% respectively. All standards and unknowns were performed in duplicate.

2.4 Histological examination The pregnant rats were randomly divided into two groups, the number of treatment group was 24 and control group was 12. After treatment as above mentioned, rats were decapitated at d 7, d 9, d 11, d 13 of pregnancy. Whole utero-conceptus complex were rapidly dissected out, fixed in 10% neutral buffered formalin, embedded in paraffin, and stained with hematovylin-eosine.

2.5 Human decidual cells culture[5] Human decidual cells were dispersed and planted $(2\text{-}3) \times 10^5$ cells/well in 0.5 ml FD medium, and supplemented with 10% BSA, penicillin 25 kU/L, streptomycin 25 g/L. Different concentration dihydroqinghaosu, which is the active metabolite of SA[6], were added respectively after 24 h incubation. All treatments were performed in 4 wells. Besides, 4 wells received media or vehicle (Me_2SO) as control group. Cells were cultured at 37℃ in 5% CO_2. Cell viability was assessed by typan blue dye exclusion.

2.6 Statistic analysis Results were expressed as $\bar{x} \pm s$ and compared by t test. LC_{50} of cultured cells and ED_{50} of contragestation were calculated as Bliss.

3 Results

3.1 Contragestational effect of SA in rats The contragestational ED_{50} of SA given *sc* on d 6-10 of pregnancy in rats was 13.81 (11.14-17.20) $mg \cdot kg^{-1} \cdot d^{-1}$ and ED_{95} was 33.06 (26.72-40.84) $mg \cdot kg^{-1} \cdot d^{-1}$. (Tab 1)

Table 1 Contragestational effect of SA given sc on d 6-10 of pregnancy in rats

Dose ($mg \cdot kg^{-1} \cdot d^{-1}$)	No. of animals	Pregnancy terminated animals NO.	$\%ED_{50}$	95% fiducial limits ($mg \cdot kg^{-1} \cdot d^{-1}$)
27.20	10	9	90	
19.20	10	7	70	
13.33	10	5	50	13.81
10.00	10	3	30	(11.14~17.20)
7.50	10	1	10	
control	10	0	0	

3.2 Effect on serum progesterone In pregnant rats, a decrease of serum progesterone in treated group was found. Compared with control groups, serum progesterone level of experimental groups declined dramatically from d 9 of gestation ($P < 0.05$) and was very significantly lower than that of control group on d 11 ($P < 0.01$). In pesudopregnant rats, serum progesterone level of the control group at d 5 and d 7 did not show any difference to that of pregnant rats. From d 9 the serum progesterone start to decline dramatically. The serum progesterone in psuedopregnant rats which treated with SA has no significant difference at d 7 or d 9 but declined dramatically at d 11 compared with the control group (Tab 2).

Table 2 Serum progesterone in pregnant and pseudopregnant rats after given *sc* SA 40 $mg \cdot kg^{-1} \cdot d^{-1}$ or 0.3% $NaHCO_3$ 0.5 $mL \cdot d^{-1}$ on d 6-10 of pregnancy or pseudopregnancy / $n = 10, \bar{x} \pm s$

Time	Serum levels of progesterone			
	Pregnant rats ($nmol \cdot L^{-1}$)		Pseudopregnant rats ($nmol \cdot L^{-1}$)	
	control	drug	control	drug
d7	133.10 ± 23.82	128.67 ± 20.15	136.14 ± 41.50	133.91 ± 74.78
d9	138.50 ± 25.65	91.72 ± 24.86*	100.36 ± 36.41	99.07 ± 24.62
dl1	147.99 ± 42.00	82.46 ± 35.06**	101.18 ± 37.91	63.69 ± 19.35#
dl3	200.78 ± 51.46	83.28 ± 40.75***	35.30 ± 15.70	10.57 ± 4.19#

Compare with control group (of pregnant rats), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Compare with control group (of pseudopregnant rats), # $P < 0.05$

3.3 Effect on uterus, decidual cells and fetus in rats In the experimental group, on d 9 of gestation, part of endo-uterus has bleeding and inflammatory cells. On d 11, its planceta was smaller than that of control group at the same time and the de-

ciduas was edema, vascular was enlarged. On d13, fetus was surrounded by RBC and inflammatory cells obviously and absorbed. In all samples, there was no obviously effect on the luteal cells on d 7 to d 11. On d 13, the luteal cells showed regression.

3.4 Effect on the viability of human decidual cells cultured in *vivtro* After human decidual cells were exposed to 5% (v/v) Me_2SO for 48 h, the viability and the morphology was unchanged *vs* media. Viability was a function of the concentration 48 h after medication. With the drug dihydroqinghaosu concentration increased, the cell viability dropped *vs* control (media and Me_2SO). A good correlation was found between cell viability and drug concentration with a linear coefficient of 0.996(Fig 1). And the morphology of the cells was greatly changed too. With the Bliss, we determined the LC_{50} of dihydroqinghaosu was 25.18 ± 3.49 mg·L^{-1}.

4 Discussion

The present study indicated that after treatment with SA on d 6-10 of gestation in rats, pregnancy was terminated. It was similar to the report[3] that SA had a contragestational activity to cause fetuses absorbed while the fetal rats survived were normal and without deformity.

In this study, SA 40 mg·kg^{-1}·d^{-1} was taken as a effective dose of terminating pregnancy in rats. The study was focused on the changes of serum progesterone and uterine decidual cells of pregnant or pseudopregnant rats after treated with SA 40 mg·kg^{-1}·d^{-1}. The results showed that after 3 d treatment with SA caused a decrease of serum progesterone level on d 9 of pregnancy. It declined 33.8% compared with the control. After 5 d treatment, serum progesterone level was 82.46 ± 35.0 nmol·L^{-1} on d 11, while that of control group was 147.99 ± 42.0 nmol·L^{-1} ($P < 0.01$). Since progesterone was so important throughout gestation[6], it showed that the mechanism of contragestative action of SA was related to its decreasing serum progesterone contents. But why did serum progesterone level decrease? According to our study, serum progesterone content on d 9 of pseudopregnancy in pseudopregnant rats treated with SA seemed no difference to that of control group, while on d 11 and d 13, it had a dramatically declined compared with control group rats. Form above we can see that the decrease serum progesterone in pregnant rats is prior to that of pseudopregnant rats. Because there are no fetuses in pseudopregnant rats, the result suggests that SA decreased the serum progesterone may not followed by its damage on ovaries but followed by its feticide effect. The results of histological examination demonstrated that after pregnant rats administered *sc* SA 40 mg·kg^{-1}·d^{-1} on d 6-10 of gestation, there were no significant difference in the structure of corpus luteum on d 11 but degraded on d 13 when the serum progesterone concentration was declined significantly. But rats in the experimental group showed smaller placentas and hyperemia in the placenta and decidua on d 9 of pregnancy, edema, degeneration and solvate of the decidual cells were seen on d 11 and the fetuses were surrounded by RBC and in inflammatory cells and absorbed on d 13. It also suggested that the regression of corpus luteum was followed by embryos damaged. The regression of luteum then caused serum progesterone declined more significantly. So we thought SA can damage fetus directly after administered.

In this study, the cultured human decidual cells exposed to the active metabolite of artesunate - dihydroqinghaosu for 48 h, with the concentration of drug increased, the viability dropped *vs* control, LC_{50} was 25.18 ± 3.49 mg·L^{-1}. It suggested that SA can damage the decidua and the decidual tissue may be one of targets of SA in terminating pregnancy action.

In summary, the results suggested that SA cause siteselective injury to decidua and uteroplacenta which inhibited the fetus growing, or damage the fetus directly, at last terminate pregnancy. According to the observations, we consider that SA should not be used to treat malaria in pregnant women.

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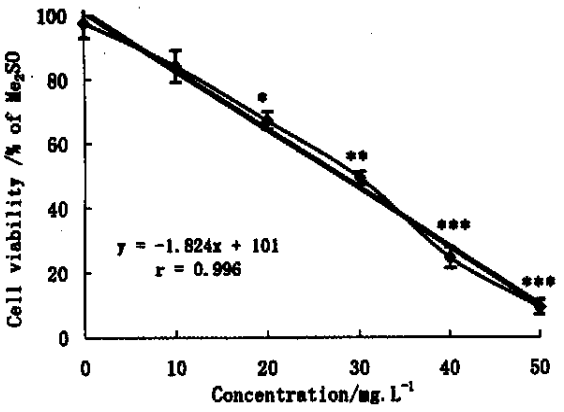

Fig 1. Viable human decidual cells in culture with dihydroqinghaosu for 48h. $n = 4$, $x \pm s$. Compared with control, * $P < 0.05$, ** $P < 0.01$ *** $P < 0.001$.

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