

Experimental Study on the Treatment of Premature Ovarian Failure by Liuwei Dihuang Pill

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Abstract: Objective: Observe the effects of Liuwei Dihuang pill on rats' premature ovarian failure. Methods: Establish rat premature senility model, and detect the effects of Liuwei Dihuang pill on p-endorphin, serum estrogen, progesterin, ovary and uterine histology. Results: Liuwei Dihuang pill can improve p-endorphin content, and increase the HEK (Human Epidermal Keratinocytes) and superficial cells in rat's exfoliated vaginal cell smear. Conclusion: Liuwei Dihuang pill has estrogen-like effects, and can condition hypothalamus-pituitary-ovary axis function, and restore ovarian function.

Key Words: Liuwei Dihuang pill; ovary premature senility; experimental research

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Premature ovarian failure (POF) refers that, women suffer from premature menopause before the age of 40, with incidence up to 1% -3% of all women[1], and often accompanied by other endocrine immune disorders, therefore, it is more difficult to treat[2-4]. Mostly women in the clinical latency period often have ovarian hypofunction and ovulatory dysfunction, giving rise to a series of symptoms, such as menstrual disorder, sterility, hypogonadotropic hypogonadism and hypogonadism, climacteric syndrome, etc. Estrogen alternative therapy is commonly applied in clinical practices, however, such therapy can not resume ovary function, and the instability of its side-effect and curative effect should not be ignored. In recent years, along with the rise of research on Liuwei Dihuang pill, to substitute Liuwei Dihuang pill for estrogen in the treatment of POF has already attracted the world's attention[15].

The experiment establishes model with rat, and through observing the regulating actions of Liuwei Dihuang pill on rat's hypothalamus-pituitary-ovary axis, rehabilitate and improve ovarian function, enhance estrogen level, adjust secretion, further explore the treatment mechanism of POF by Liuwei Dihuang pill, and provide a reliable basis for theoretical and experimental basis for treating POF by Liuwei Dihuang pill.

1 Data and Methods

1.1 Experimental Animal: Only 30 female Wistar rats at 60-day-old, with body weight of 180g-200g, provided by Shanxi Medical University.

1.2 Drugs and Reagents: Liuwei Dihuang pill from Shanxi Chinese Medicine Factory production (Lot: 000212); hydrocortisone from Chengdu Lihua Pharmaceutical Factory production, 10mg/ml (Lot: 990208); and, diethylstilbestrol from Shanghai Hengshan Pharmaceutical Co., Ltd. Production (Lot: 000201).

Estradiol (E:) radioimmunoassay kit purchased from Tianjin Depew Diagnosis Products Co. Ltd; luteal phase (P) radioimmunoassay kit purchased from the Nuclear Medicine Technology Center of the Chinese Academy of Medical Sciences; and, B-endorphin radioimmunoassay kit purchased from the Navy Technology Center RIA.

1.3 Apparatus and Equipment: electronic thermometer: DT-ITB type; electronic balance: JD2000-2; Y-counter:

GAMMA-C12.

1.4 Establishment of Model and Experimental Modelling Method: refer to the literature [6]. Conventionally feed 30 rats 1w, and randomly divide them into 5 groups: normal control group (Group A), model group (B group), group treated with small dosage of Liuwei Dihuang pill (C group), group treated with big dosage of Liuwei Dihuang pill (D group), western medicine group (E group), and 6 rats for each group. Group A is still normal fed, without any treatment; And, Group B have 20mg/kg hydrocortisone (hereinafter referred to as hydrogen) injected by intramuscular; The hydrogen treatment on Group C is the same as Group B, with 2g/kg intragastric administration of Liuwei Dihuang pill at the same time; the hydrogen treatment on Group D is the same as Group B, with 4g/kg intragastric administration of Liuwei Dihuang pill at the same time; and, the hydrogen treatment on Group E is the same as Group B, with 0.04mg/kg diethylstilbestrol intragastric administration at the same time. The above treatment is once a day, and for 2 nights continuously.

1.5 Observation Indicators: (1) Only observe every rat for its changes in estrum of epithelial exfoliated cells from externalia and cunt, and carry out regular stain smear, HE stain and cytological examination every afternoon. (2) Upon the drug withdrawal after a continuous oral administration of each group 2w, weigh and, determine the B-endorphin, estrogen and progesterone levels of rats in anestrus by radioimmunoassay kit instructions. Take blood from the rim of rat's eye after light anesthesia with ether by vein blood sampling, suck out serum with centrifuge (3000r/min) for 10min, store in refrigerator at -20°C, and test P and E2 batch by batch. While keep B-endorphin sample, it requires 1% heparin and trasyolol for anticoagulation, rapidly centrifugalize at low temperature (4°C), and take and preserve plasma at low temperature (-20°C) for further test purpose. (3) After blood sampling, kill the animals by the decapitation method, remove uterus and ovary, remove fat and other tissues, slightly swab with filter paper, separately put into a scale, and precisely weigh. (4) Fix uterus and ovary fixed, get them embedded, sliced up and treated by HE stain, and then, carry out histological examination. (5) Separately measure rats' rectal temperature on the 3rd, 6th, 9th and 14th days after the intragastric administration. While measuring, insert electronic thermometer into rat's anus to 2cm, determine rectal temperature (Accuracy: 0.1°C). Once the temperature does not rise for 10 seconds after thermometer is inserted into anus, the thermometer will make a sound.

Note: Read and record the values.

2 Results

2.1 Influences of Rat's Exfoliated Vaginal Cell Smear: Rat's sexual cycle is generally 4~5 days. In the first night after administration, except Group E's cycle has varying degrees of extension(5-6 days), the rest groups have a same cycle. It reflects that Group B and Group C have cycle disorders, with varying periods. The cycles of Group D and Group A is basically stable, for 4-5 days. In the 2nd night after the Intra gastric administration, Group A had no significant change in cycle, still for 4-5 days; and, the rest groups have obvious changes, showing Group D's cycle extended to 6-7days, Group E's cycle extended to 7~8 days, Group C is basically tend to normal, for 4~5 days, and Group B's cycle is still irregular. The results show: Group D, Group E and Group A have a significant difference in their highest values of keratinizing epithelial cells and the those of superficial cells($P<0.05$), Group C and Group A have no significant difference($P<0.05$), Groups C, D, E and B have significant statistical difference($P<0.05$).

2.2 Changes in Serum Estrogen, Progesterin Level and B-Endorphin Content: see Table 1.

Table 1 Comparison between All the Groups in E2, P and B-EP($\bar{x} \pm s$)

Category	n	E2	P	B-EP
Group A	6	15.06±1.02	10.19±0.28	310.69±21.34
Group B	6	7.16±0.88	6.13±0.49	217.79±18.03
Group C	6	9.73±0.66	7.31±0.44	292.79±16.39
Group D	6	14.91±1.19	9.04±0.86	306.60±23.63
Group E	6	15.00±1.05	6.49±0.59	306.24±25.36

2.3 Morphological Changes: morphology characteristics of ovarian tissue(light microscope: 20*10): Group A and Group C show a very few primordial follicles, primary follicles, secondary follicles and mature follicles, and more corpus luteums. Some corpus luteums appear fresh visible luteal blood, with few interstitials and few interstitial glands. Group B shows primordial follicles, more atretic follicle, more interstitial glands and old

corpus luteumss, without primary follicle, secondary follicle or mature follicle. Group D and Group E show primordial follicles and a large number of primary follicles, secondary follicles and nearly mature follicles, more corpus luteums, less interstitials, and rare interstitial glands.

Uterus Morphology by Light Microscopy(20x10): Group A and Group C shows normal endometrium, with intima thickness up to 2 / 3 of the thickness of uterine wall, epidermis giving priority to secretory cells, cytoplasm containing substantial secretory vacuoles, laminae propria having a large number of uterine glands, large gland cavity, cavity having secretions inside, loose connective tissue, having rich capillaries, small arterials and venas. Group B shows thin endometrium, with thickness up to 1 / 3 of uterine wall thickness, epidermis giving priority to column cells, few secretory vesicles in cytoplasm, lamina propria with connective tissue widely distributed, less uterine glands, and small glandular cavity. Group D and Group E show thick intima, with thickness up to 1/3—2/3 of uterine wall, simple columnar epithelium, more secretory cells, laminae proprialoose connective tissue, substantial uterine glands, and rich blood vessels.

3 Discussion

POF belongs to many categories of traditional Chinese medicine, such as "depleted blood", "hematodysphasia with blood stasis", "amenorrhea", etc., and through identifying etiology according to differentiation of symptoms and signs, we may found it is always indispensable for the root of premature senility of kidney-QI and gives priority to kidney-YANG deficiency.

B-EP is an important regulating factor for female reproductive endocrinology. Hypothalamus is rich in B-EP, having an important physiological role in pituitary, central nervous system regulating body temperature and eating central nervous system [9]; and, at the same time, research found that, B-EP is closely related to the hypothalamus-pituitary-adrenal gland axis and the hypothalamus-pituitary-ovary axis [7,8]. The function of this axis is that of "kidney" in the traditional Chinese medicine, and it's confirmed that this is the essence of "kidney" in the TCM. The experimental research results show that the plasma B-EP content of both of the rate model in hydrocortisone and ovariectomized rat were significantly decreased. Note: Changes in B-EP is related to the occurrence of clinical symptoms induced by kidney-yang deficiency and oophorectomy. As for the reasons for the decline of B-EP, It may be associated with hydrocortisone substantially used to suppressing ovarian estrogen and progesterone secretion and changes in estrogen and progesterone caused by surgery. The plasma B-EP content of the model rat treated by Liuwei Dihuang pill and western medicine is close to that of the Group A. It shows that Liuwei Dihuang pill can get B-EP content resume normal. Note: B-EP may be involved in the function of gonadal axis, in particular, having promoting, regulating and improving ovarian function.

The maturity of vaginal epithelium is proportional to the in vivo estrogen level. The main functions of estrogen is to get epithelial proliferation and cell maturity. Its incidence is generally based on keratinocytes index: a).When the estrogen level increases, vaginal cells have more keratinocytes and pyknotic nucleus phenomenon. When estrogen level drops, cell smear has small round, oval and nuclear-osteoporosis middle and bottom cells. Liuwei Dihuang pill can enhance rat's estrogen level, causing HEK (Human Epidermal Keratinocytes) and superficial cells increased.

Traditional Chinese medicine considers, kidney storing essence and controlling reproduction is the congenital foundation. In case of kidney-QI deficiency and kidney-essence sufficiency, the kidney has normal function of controlling reproduction. Ovum is the sperm in the possession of kidney, kidney-essence is its material foundation, and kidney-YANG is the power for its growth. If the kidney-essence is sufficient, it shows the normal development of ovum. Yang governs movement, and the motive power released by the maturity of ovum comes from the agitation of kidney-Yang. Kidney governs hibernation, and controls opening and closing. Kidney controls hiding, and to control opening and closing is the functional performance of kidney-YANG. On the one hand, "Yang is secured by defending exterior", and kidney-YANG guarantee not to open but close before ovulation, and to exert its functions to control hibernation, closing and hiding; kidney stores essence without diarrhea, Only when the kidney-essence is sufficient will ovum be mature; on the other hand, Yang governs movement, the gasification of kidney-YANG get kidney temporarily open during the ovulation period, and the kidney-Yang gasified acts as an internal power to agitate for mature ovulating. If the kidney-YANG is deficient, the hiding function will become dysfunctional. On the one hand, once the kidney-yang is not close properly, folliculi will not develop into maturity. On the other hand, if it is not open properly, it will fail to act as an inherent power to instigate for ovulation. It shows that, Liuwei Dihuang pill can improve and enhance ovarian function, further promote and improve the hypothalamus-pituitary-gonadal axis function, and achieve the goal of treating POF.

In conclusion, the role of Liuwei Dihuang pill is not a simple substitute for estrogen or single-factor intervention, but a recovery of the functions of ovary and hypothalamus-pituitary-gonadal axis through improving the endocrine system function, and, the effects of Liuwei Dihuang pill on ovary are not single or independent, but multi-faceted and integrated. It is considered that the possible mechanisms is: (1) Through conditioning the hypothalamus-pituitary-ovary axis function, restore ovarian function. (2) Liuwei Dihuang pill has estrogen-like effects.

References

[1]. Christin-Maitres, Bouchard P. *Ann Endocrinol*, 1999,60(2): 118-122.
 [2]. Layman LC et al. *Fertil Steril*, 1993, 60: 852-857.

[3]. Hock A, Schoemaker J, Drexhage HA. Premature ovarian failure and ovarian autoimmunity. *Endocr Rev* 1997, 18: 107-114.
 [4]. Wheeler CA. Premature Ovarian failure: Uterine and Ovarian Pathogenesis. *Reprod Med*, 1995,78: 130-131.
 [5]. Luo Pushu, Liu Chunxia and Shi Manhong, *TCM Dialectical Treatment of 106 Ovarian Hypofunction Patients*, Chinese Journal of Integrated Traditional and Western Medicine, 1998, 1s(2): 119-120.
 [6]. Lv Xiaoduo, Lu Ruixia and Ye Xueqing, Yang-Deficiency Model Animals' Changes in Hypothalamus β -Endorphin and Effects of Drugs Reinforcing the Kidney Yang[J]. *Journal of Traditional Chinese Medicine*, 1994, 35 (10) : 619-620
 [7]. Wang Qin, *Endorphin and Gynecological Endocrine*, *Acta Academiae Medicinae Jiangxi*, 1988, 2s(3): 91-94.
 [8]. Shen Weibin, Wang Hong and Zhang Chongli, *Mechanism of P-Endorphin in the Negative Feedback Regulation of Estrogen*, *Acta Zoologica Sinica*, 1990,36(3):286-291.

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