

## • 实验研究 •

# 补肾补脾中药对大鼠甲状腺、肾上腺切除后卵巢功能减退的补偿治疗作用

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**内容提要** 大鼠肾上腺、甲状腺切除后，卵巢重量减轻，卵巢内 HCG/LH 受体功能降低。补肾中药(附子加熟地)能使上述动物卵巢增重，HCG/LH 受体功能提高。补脾中药(四君子汤)或者单用附子或熟地则无此作用。可能是补肾中药提高了卵巢对垂体促性腺激素的反应能力，从而使卵巢功能恢复。

中医学认为，肾为先天之本，肾主藏精，与人体生殖功能有密切关系。脾为后天之本，水谷精气必须通过脾的运化，才能营养机体，维持机体正常生殖功能。中医妇科临床很重视补肾作用，由于肾与脾乃先天和后天关系，补肾常兼补脾。而近代研究表明，肾的功能与肾上腺皮质有密切关系<sup>①</sup>，补肾能增强肾上腺皮质功能。而甲状腺功能则与机体新陈代谢有密切关系。由此设想，当切除肾上腺或甲状腺后造成卵巢功能减退，是否通过中药补肾或补脾有补偿治疗作用，从而为临床应用提供参考。

## 材料与方

一、实验动物及分组：选用雌性大白鼠，体重 120~150g (实验结束时体重 150~200g)。在手术前一周内，每天阴道涂片检查动情周期，选用出现动情期的大白鼠用于实验。所有实验动物在乙醚麻醉下，摘除大白鼠肾上腺或甲状腺。切除甲状腺动物，术前及术后 1~4 周每周测定空腹时耗 O<sub>2</sub> 量<sup>②</sup>，以术后四周耗 O<sub>2</sub> 量减少 30% 以上作为甲状腺功能低下的指标，符合此条件的动物选做实验。分组：(1)正常假手术组。(2)切除肾上腺或切除甲状腺组。(3)切除肾上腺或切除甲状腺加补肾中药(附子加熟地)组。(4)切除肾上腺或切除甲状腺加补脾中药(四君子汤)组。另外切除肾上腺的大白鼠单独服用附子或熟地煎剂作了对照观察。

## 二、药物与试剂：

1. 中药：由我院附属中医院药房提供，经中药教研组鉴定。实验用附子(Radix Aconiti Praeparata)，熟地(Radix Rehmanniae)，党参(Radix Codonopsis Pilosulae)，白术(Rhizoma Atractylodis Alba)，茯

苓(Poria)，甘草(Radix Glycyrrhizae)。

2. 试剂：放射受体测定所用的<sup>125</sup>I-人绒毛膜促性腺激素(<sup>125</sup>I-HCG)由上海生物制品研究所提供，并用 Tris-HCl 缓冲液(0.04mol, pH7.4, 内含 0.005mol 的 MgSO<sub>4</sub>) 配制而成 17.2ng/ml 的溶液。由于溶液在放置过程中脱落碘增加，故每次试验时需测定其碘化纯度(按照：Leidenberger, F 及 Reichert, L.E.Jr 方法进行)<sup>③</sup>。

3. 中药制剂及服药时间：补肾中药(附子加熟地)，补脾中药(党参、白术、茯苓、甘草)。按组方生药的总重量(克数)，第一煎每克生药加水 7 ml，在电炉上加热煎煮，至沸后 30 分钟(附子先用武火煎煮 40 分钟)，再用六层纱布过滤并收集滤液于瓶中。第二煎每克生药加水 3 ml，与第一煎方法同样煎煮。合并两次滤液于水浴上蒸发，最后浓缩为 1g 生药/ml，贮存于冰箱中待用。按 1ml/100g 体重/日，灌胃给药，连续五天，对照组服等量生理盐水。于最后一次灌药的第二天上午，处死动物，迅速摘出双侧卵巢，称重，测定卵巢人绒毛膜促性腺激素/黄体生成素(HCG/LH)受体功能。

三、卵巢 HCG 受体结合活力测定：基本按照 Catt KJ<sup>④</sup> 和 Lee CY<sup>⑤</sup> 所介绍方法进行。将卵巢投入冰冷的 Tris-HCl 缓冲液中，用玻璃匀浆器在冰水浴中作成匀浆，稀释度为 1:10 (W/V)，取卵巢组织匀浆 0.1ml (内含卵巢组织 10mg)，加<sup>125</sup>I-HCG 0.1 ml (含<sup>125</sup>I-HCG 1.72ng)，加温培液(含 0.1% 牛血清白蛋白的 Tris-HCl 缓冲液)至最终体积为 1ml。在 37°C 水浴下温育 30 分钟，立即加冰冷缓冲液 2ml 以终止反应。将此培育物测定总放射脉冲数/分，然后通过醋酸纤维微孔滤膜(孔径 0.45μm，上海第十制药

厂生产,用前置4%牛血清白蛋白中浸泡半小时)抽滤,再用2ml冷的缓冲液通过滤膜抽滤,以洗去残留未结合碘。用r-计数器(FH-408自动定标器,国营二六一厂)测定滤膜之放射活性(脉冲数/60'')按下式计算HCG总结合量:

$$\frac{\text{滤膜之脉冲数(结合)}}{\text{培养液总脉冲数} \times \text{碘化纯度}} \times 1720 \text{pg} \\ = \text{HCG}(\text{pg}/10\text{mg 卵巢})$$

为了测定卵巢匀浆HCG的非特异结合,在每一测定管同时作一平行测定管,其中加入过量非标记HCG(2,000ng),以抑制特异结合,求得非特异结合量,从总结合量中减去非特异结合量,即为卵巢受体对HCG之特异结合量,以HCG(pg/10mg 卵巢)表示(见表1、2)。

为了测定卵巢HCG受体的数量及其对HCG的亲合力,需对同一组织匀浆作一系列结合试验。向各测定管加入四个不同剂量之 $^{125}\text{I}$ -HCG,测定标记HCG结合量(同时平行测定非特异结合量),以各管加入 $^{125}\text{I}$ -HCG量为横座标,相应特异结合量为纵座标,作出特异结合曲线。并且作Scatchard分析,作图求出每毫克卵巢组织之最大结合量(Bmax)以 $10^{-15}\text{M}$ /10mg 卵巢表示,代表卵巢HCG受体的数目,以及解离常数(Kd),以 $10^{-10}\text{mol/L}$ 表示,代表卵巢HCG受体亲和力(6,7)(附图)。

## 实 验 结 果

结果表明 切除肾上腺或甲状腺后大白鼠卵巢重量,卵巢对 $^{125}\text{I}$ -HCG结合量,与正常假手术组相比,明显降低,P值分别 $<0.05$ 及 $<0.01$ 。切除肾上腺、甲状腺后,分别给予补肾中药(附子加熟地)煎剂,卵巢重量,卵巢对 $^{125}\text{I}$ -HCG结合量与切除肾上腺或甲

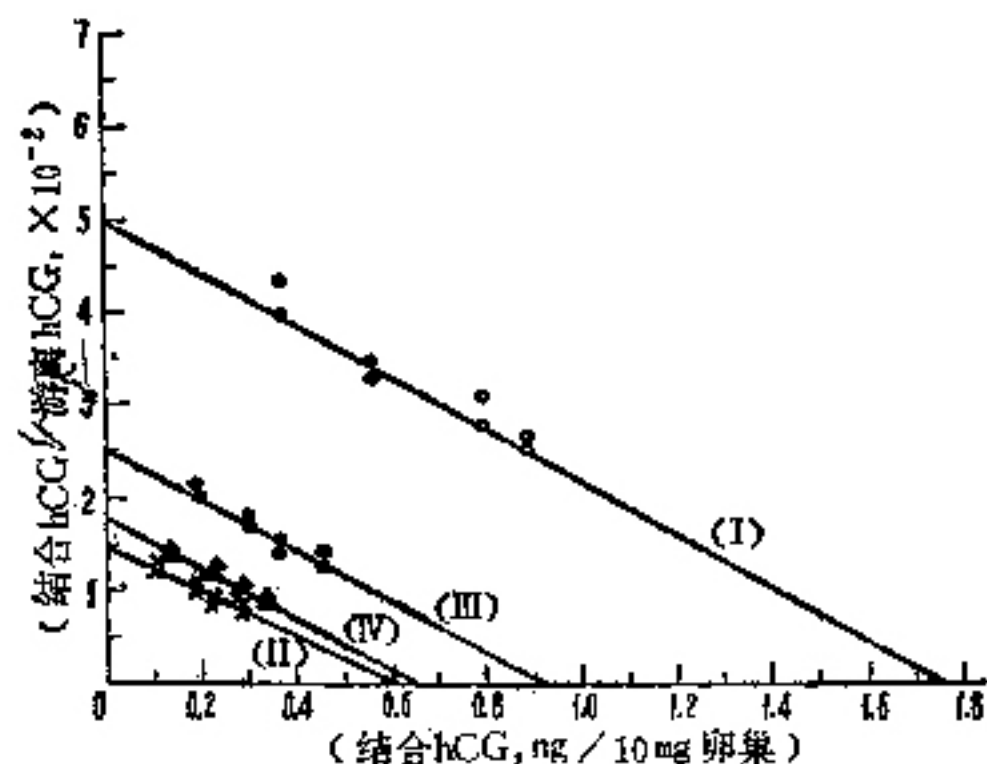
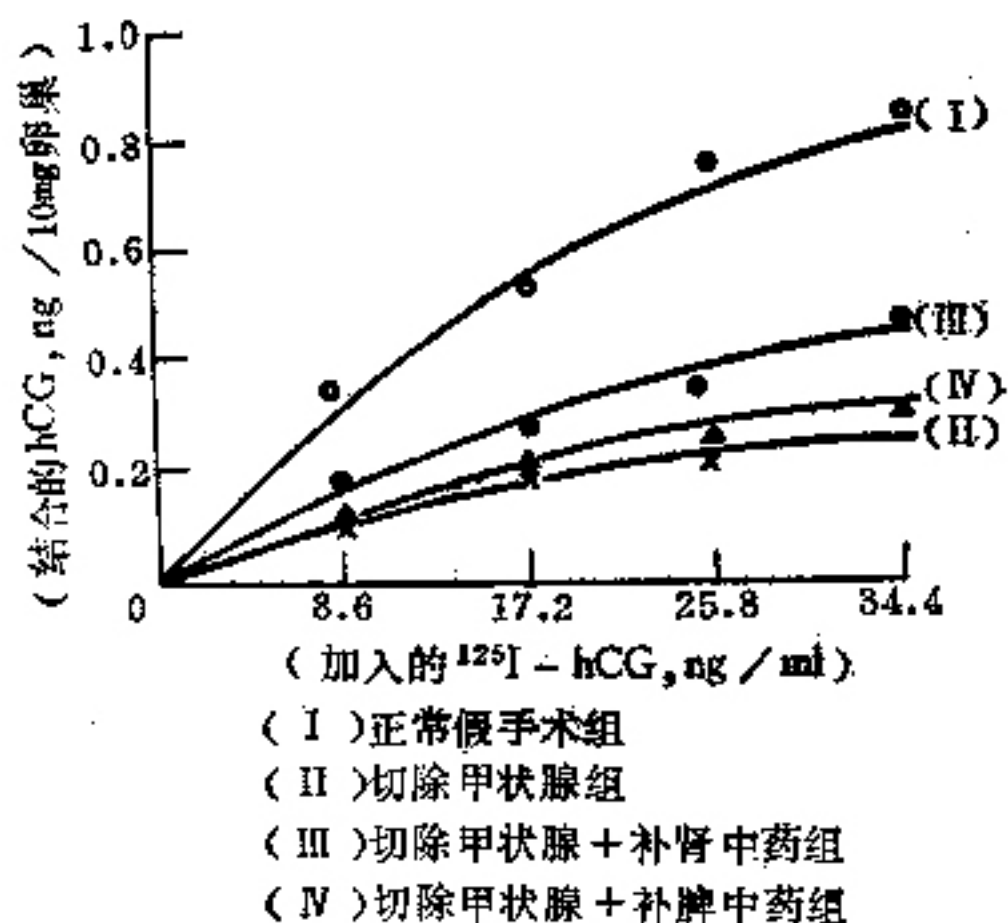
表1 补肾补脾中药对肾上腺切除后卵巢HCG/LH受体功能影响 (M±SD)

| 组 别             | 动物数 | 卵巢重量<br>mg/100g体重 | 卵巢对 $^{125}\text{I}$ -HCG<br>结合量<br>pg/10mg卵巢 |
|-----------------|-----|-------------------|-----------------------------------------------|
| 正常假手术组          | 15  | 34.20±1.20        | 190.50±5.01                                   |
| 切除肾上腺组          | 15  | 26.80±2.24        | 115.20±6.31                                   |
| 切除肾上腺+<br>补肾中药组 | 5   | 32.34±3.90        | 171.26±8.40                                   |
| 切除肾上腺+<br>补脾中药组 | 5   | 28.00±1.46        | 117.84±1.54                                   |
| 切除肾上腺+<br>附子组   | 5   | 28.40±2.30        | 123.50±2.69                                   |
| 切除肾上腺+<br>熟地组   | 5   | 27.02±1.80        | 116.34±2.40                                   |

表2 补肾补脾中药对甲状腺切除后卵巢HCG/LH受体功能影响 (M±SD)

| 组 别             | 动物数 | 卵巢重量<br>mg/100g<br>体重 | 卵巢对 $^{125}\text{I}$ -HCG<br>结合量<br>pg/10mg<br>卵巢 | 对 $^{125}\text{I}$ -<br>HCG最<br>大结合量<br>( $10^{-15}$<br>M/mg) | Kd 值<br>( $\times 10^{-10}$<br>M/L) |
|-----------------|-----|-----------------------|---------------------------------------------------|---------------------------------------------------------------|-------------------------------------|
| 正常假手术组          | 5   | 36.03<br>±1.52        | 169.20<br>±10.17                                  | 3.81                                                          | 7.56                                |
| 切除甲状腺组          | 5   | 28.87<br>±2.10        | 94.20<br>±6.53                                    | 1.32                                                          | 9.06                                |
| 切除甲状腺+<br>补肾中药组 | 5   | 41.66<br>±3.73        | 161.00<br>±7.97                                   | 2.01                                                          | 7.24                                |
| 切除甲状腺+<br>补脾中药组 | 5   | 33.44<br>±2.16        | 111.60<br>±9.54                                   | 1.43                                                          | 8.70                                |

状腺组相比,显著地增高,P值分别 $<0.05$ 、 $<0.01$ 。而与正常假手术组无明显差异,P值皆 $>0.05$ 。说明



附图 卵巢HCG/LH受体对 $^{125}\text{I}$ -HCG的特异结合及Scatchard分析

补肾中药(附子加熟地)能使卵巢重量恢复接近正常,卵巢对 $^{125}\text{I}$ -HCG结合量近似于假手术对照组。

大白鼠摘除肾上腺或甲状腺后,分别给予补脾中药(四君子汤)或单独用附子或熟地煎剂,卵巢重量,卵巢对 $^{125}\text{I}$ -HCG结合量与切除肾上腺或切除甲状腺组相接近, $P$ 值均 $>0.05$ ,但仍然显著低于正常假手术组, $P$ 值分别 $<0.05$ 、 $<0.01$ 。说明补脾中药(四君子汤)或者单用附子或熟地煎剂对切除肾上腺或切除甲状腺引起的大白鼠卵巢重量减轻,卵巢对 $^{125}\text{I}$ -HCG的结合量降低无纠正作用。

实验结果还表明:大白鼠甲状腺切除后,卵巢对 $^{125}\text{I}$ -HCG的最大结合量,与正常假手术组相比,明显下降, $K_d$ 值则增高。给予补肾中药(附子加熟地)煎剂,则上述切除甲状腺的影响可以补偿。 $B_{\max}$ 有所提高, $K_d$ 值下降接近正常。

## 讨 论

正常卵巢功能需要肾上腺、甲状腺分泌激素作用。已知切除大白鼠肾上腺,生殖器官萎缩,动情周期改变或停止<sup>(8,9)</sup>。甲状腺机能不足,卵巢萎缩,动情周期不规则,卵泡发育停滞,不排卵,附性器官退缩<sup>(10)</sup>。我们切除大白鼠肾上腺、甲状腺后,卵巢重量减轻,卵巢HCG/LH受体功能降低,从受体水平表明了甲状腺、肾上腺对性腺功能的影响。其原因可能是失去肾上腺皮质激素和甲状腺激素的支持,卵巢发生退缩,使卵巢受体数量减少。也可能是卵巢对垂体促性腺激素反应能力低下,使卵巢功能低下,卵巢HCG/LH受体功能降低。

补肾中药(附子加熟地)能使切除肾上腺或甲状腺大白鼠卵巢增重,卵巢内HCG/LH受体功能恢复。黄华楼等<sup>(11)</sup>研究认为补肾中药具有肾上腺皮质激素样作用,并把这种作用作了两种可能解释(1)促进垂体—肾上腺皮质系统分泌较多的皮质激素。(2)可能补肾中药本身含有类似皮质激素物质或者有皮质激素样物质前体,在体内转变为皮质激素。李德兴等<sup>(12)</sup>报道附子煎剂能增加大鼠尿中17-酮类固醇的排泄,降低肾上腺内抗坏血酸含量,减少末梢血液中嗜酸性白细胞,认为附子煎剂对垂体—肾上腺皮质系统有兴奋作用。如果认为补肾中药(附子加熟地)仅只有上述作用。那就无法解释大白鼠甲状腺切除给予补肾中药(附子加熟地)也能使卵巢增重,卵巢HCG/LH受体功能恢复。可见补肾中药还有其他的作用机制。我

们的实验结果还表明:补肾中药(附子加熟地)不但使卵巢HCG/LH受体位数有所提高,同时也使卵巢HCG/LH受体 $K_d$ 值降低接近正常。说明补肾中药通过提高机体反应性提高卵巢对垂体促性腺激素的反应能力,改善切除肾上腺、甲状腺后大白鼠下丘脑—垂体—性腺轴功能,使卵巢重量增加,卵巢HCG/LH受体功能提高。

在本实验中,补脾中药(四君子汤)对肾上腺、甲状腺切除引起的卵巢重量减轻,卵巢HCG/LH受体功能降低未表现有纠正作用。是否补肾补脾中药合用能表现有作用,尚待进一步证实。单独用附子或熟地也未表现有作用,所以附子和熟地在临床上常配伍使用,可能有一定道理。

(蒙杨鸿志同志技术协助,谨致谢意。)

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## The Effect of "Huo Xue Hua Yu" Drugs on Right Ventricular Function and Blood Rheology in Chronic Cor Pulmonale

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Injection of Radix Salviae miltiorrhizae was given intravenously to 26 chronic cor pulmonale patients (23 men and 3 women) aged  $51.2 \pm 5.4$  years in average, after the subsidence of acute infection. Their right ventricular function and blood rheology were improved significantly as follows: PEP time reduced from  $0.133 \pm 0.018$  second to  $0.118 \pm 0.017$  second; RVET time increased from  $0.292 \pm 0.019$  second to  $0.324 \pm 0.020$  second; PEP/RVET ratio decreased from  $0.460 \pm 0.083$  to  $0.395 \pm 0.084$ ; Q-Z time reduced from  $0.187 \pm 0.014$  second to  $0.176 \pm 0.016$  second. The whole blood viscosity and red corpuscle electrophoresis were also significantly improved ( $P < 0.05$ ).

The study shows that "Huo Xue Hua Yu" drugs or drugs with action to activate blood circulation and relieve blood stasis can increase the myocardial contraction and reduce the after load of the right ventricle. There is a positive correlation between PEP time, whole blood viscosity and red corpuscle electrophoresis. The increase of whole blood viscosity and hematocrit may increase the pulmonary arterial pressure. (Original article on page 220)

## The Treatment of Chronic Bronchitis with Tan Chuan Jing ( 痰喘净 ) —A Clinical Study of 300 Cases and Pharmacological Experiment

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Tan Chuan Jing is a preparation consisting of volatile oil of Fructus Phellodendron amurense ( 黄柏果 ) and extract of *Sophora flavescens* ( 苦参 ).

Phenol red tests revealed marked expectorant action of Tan Chuan Jing in mice. In guinea pig, the preparation showed evident spasmolytic action on tracheal spasm induced by histamin in vivo, but no antitussive effect was observed. LD<sub>50</sub> of the preparation was  $2.969 \pm 0.172$ g/kg for mice; subacute toxicity tests showed that Tan Chuan Jing (po, 1 100mg/kg) delayed the growing of rats, but exerted no influence on routine blood test, CPT and BUN.

300 patients were treated with Tan Chuan Jing, each taking 2 capsules, two times a day during the coldest season. The patients treated with this preparation had a total effective rate of 97% with a prominent improvement rate of 65%. Tan Chuan Jing has marked expectorant, antiasthmatic and antiphlogistic effects, however, it has little antitussive effect. This preparation is applicable to both types of chronic bronchitis, simple and asthmatic, but it is more effective in treating the latter. Clinically, it showed no apparent toxic side effect.

(Original article on page 222)

## The Compensative Effects of Chinese Drugs Invigorating Kidney or Spleen on Disturbed Ovarian Function of Adrenalectomized or Thyroidectomized Rats

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The weight of ovary and its binding capacity to <sup>125</sup>I-HCG of adrenalectomized adult female rats were significantly decreased as compared with the levels of sham-operation control rats. However, if the adrenalectomized rats were treated with Chinese drugs invigorating the kidney (Mankshood plus prepared Radix Rehmanniae) for five consecutive days, the weight of ovary and its binding capacity to <sup>125</sup>I-HCG returned nearly to the levels of sham-operation control group. If they were treated with Chinese drugs invigorating the spleen (Si Jun Zi Tang ( 四君子汤 )) no effect was observed.

One month later the female rats were thyroidectomized, and those whose oxygen consumption was decreased by more than 30% were used for study. In these animals the weight of ovary, its specific capacity of binding <sup>125</sup>I-HCG, and the maximum <sup>125</sup>I-HCG binding (Bmax) were decreased as compared with the levels of the sham-operation control group, while the Kd value was increased. In those animals treated with Chinese drugs invigorating the kidney (Mankshood plus prepared Radix Rehmanniae, 1gm crude drug/100gm body wt.), the weight of ovary and the specific binding capacity to <sup>125</sup>I-HCG increased, the Kd value decreased and the Bmax slightly increased, therefore, the effects of thyroidectomy can be compensated.

The results obtained suggested that in adrenalectomized or thyroidectomized rats the function of HCG/LH receptor in ovary was decreased, which could effect the function of sexual gland. Chinese drugs invigorating kidney could improve the function of HCG/LH receptor in ovary, thus the responsibility of ovary to gonadotrophin was increased. This problem merits further study. (Original article on page 227)