

证的研究

实热证、虚热证造模大鼠甲状腺超微结构及功能对比研究

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摘要 观察实热证、虚热证大鼠血清三碘甲状腺原氨酸(T_3)、四碘甲状腺原氨酸(T_4)、反三碘甲状腺原氨酸(rT_3)和促甲状腺激素(TSH)浓度及甲状腺结构改变。用中药灌服大鼠,形成实热证、虚热证模型,运用放射免疫测定和电镜技术观察。结果,虚热造模组血清 T_3 、 T_4 水平显著升高, rT_3 水平呈下降趋势;虚热造模组甲状腺滤泡上皮比实热造模组改变明显。表明甲状腺激素和甲状腺超微结构改变可作为热证实虚比较的重要依据。

主题词 实热/病理学 虚热/病理学 甲状腺/超微结构

中医学认为“阳盛则外热”、“阴虚则内热”,热证有虚实两种不同状态。据文献报道,用温热药灌喂动物形成的热证模型大鼠内分泌变化以甲状腺功能变化为主,血中三碘甲状腺原氨酸(T_3)有升高趋势,甲状腺组织病理呈功能亢进象^[1],而对实热证、虚热证两种不同状态的甲状腺超微结构和功能变化方面的实验研究,目前国内外尚少见报道。因此,本实验观察了热证造模大鼠的甲状腺结构和血清 T_3 、四碘甲状腺原氨酸(T_4)、反三碘甲状腺原氨酸(rT_3)、促甲状腺激素(TSH)浓度的变化,并与治疗后进行比较,以便寻求热证的本质以及实热证、虚热证的区别和联系。

1 材料和方法

1.1 实验动物及分组:实验动物全部由本校实验动物中心提供,采用 Wistar 系雌性大白鼠 62 只,体重 180~220g,月龄 2~3 月,随机分为 5 组。I 组:正常对照组(11 只);II 组:实热造模组(10 只);III 组:虚热造模组(15 只);IV 组:实热治疗组(13 只);V 组:虚热治疗组(13 只)。

1.2 药物及试剂:所有中药材均从本附院药剂科购进,经本院中药研究室鉴定,确定无伪,煎煮 2 次,药液浓缩至 1g/ml 生药。泻心汤按《金匱要略》配方(1g/ml 生药,大黄:黄连:黄芩为 2:1:1),六味地黄汤按《小儿药证直诀》配方(1g/ml 生药,熟地黄:山茱萸:山药:泽泻:茯苓:牡丹皮为 8:4:4:3:3:3)。

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T_3 、 T_4 、 rT_3 和 TSH 试剂盒由北京北方生物技术研究所提供。

1.3 动物造模方法:(1)实热证、虚热证组模型方法参见文献^[2,3],实热造模组每鼠每日灌以 4ml 党参、黄芪水煎剂(1g/ml 生药,党参:黄芪为 1:1),虚热造模组每日灌以 4ml 附子、干姜、肉桂水煎剂(1g/ml 生药,附子:干姜:肉桂为 1:1:1),2 周造模成功后,继续每日灌以 4ml 生理盐水 2 周,共 4 周;(2)实热证治疗组:为实热造模成功后,每鼠每日灌胃 4ml 泻心汤中药煎剂 2 周,共 4 周;(3)虚热证治疗组:为虚热造模成功后,每鼠每日灌胃 4ml 六味地黄汤中药煎剂 2 周,共 4 周;(4)正常对照组:正常饮水,普通块料喂养,每鼠每日灌胃 4ml 生理盐水,共 4 周。

1.4 血清激素测定:各组大鼠眼球采血 2ml,分离出血清,用放射免疫分析法检测,测量仪器为 BECKMAN DP5500 型 γ 计数器。分别测定血 T_3 、 T_4 、 rT_3 及 TSH。

1.5 透射电镜标本制备:将取血后的动物断头处死,迅速取一小块甲状腺组织,按电镜制样常规处理标本^[4],AO 超薄切片机切片,置 JEM-1200EX 透射电镜观察。

1.6 统计学处理:多组间比较采用方差分析,各组间两两比较用 q 检验。

2 结果

2.1 各组大鼠 T_3 、 T_4 、 rT_3 和 TSH 水平及统计学结果:其中虚热造模组的 T_3 、 T_4 均明显高于正常对照组(q 值分别为 3.60、4.29, $P<0.05$ 、 $P<0.01$), rT_3 有所下降(q 值为 3.50, $P<0.05$),虚热治疗组 T_3 与对照组差异有显著性(q 值为 3.82, $P<0.01$),而实热造模组 T_3 、 T_4 、 rT_3 和 TSH 水平变化无统计学差异($P>0.05$);经 2 周治疗后,实热造模组与治疗组、虚热造模组与治疗组比较 rT_3 有所回升,差异有显著性(q 值均为 3.13, P 均 <0.05),而 T_3 、 T_4 和 TSH 无显著改变。见附表。

2.2 透射电镜观察(图略):正常对照组:甲状腺滤泡上皮细胞低矮,呈扁平状,游离面有较多微绒毛伸入滤泡腔;核为卵圆形,位于中央,染色质分布均匀;胞浆内

附表 各组大鼠血清 T_3 、 T_4 、 rT_3 及 TSH
测定结果比较($\bar{x} \pm s$)

组别	鼠数	T_3 (nmol/L)	T_4 (nmol/L)	rT_3 (nmol/L)	TSH(mU/L)
正常对照组	11	0.94 ± 0.23	48.27 ± 21.68	0.42 ± 0.06	2.74 ± 0.97
实热造模组	10	1.27 ± 0.37	52.62 ± 23.99	$0.39 \pm 0.08^{\Delta}$	3.44 ± 1.70
虚热造模组	15	$1.38 \pm 0.55^*$	$73.27 \pm 11.28^{**}$	$0.40 \pm 0.11^{\Delta}$	2.64 ± 1.31
实热治疗组	13	0.99 ± 0.45	67.51 ± 18.44	0.47 ± 0.07	3.08 ± 1.10
虚热治疗组	13	$1.34 \pm 0.30^{**}$	67.04 ± 22.81	$0.49 \pm 0.10^*$	2.62 ± 0.86

与正常对照组比较, * $P < 0.05$, ** $P < 0.01$; 与虚热治疗组比较, $\Delta P < 0.05$; 与实热治疗组比较, $\Delta P < 0.05$

有轻度扩张的粗面内质网;高尔基复合体位于核旁,发育较好;线粒体短杆状,数量不多,并有少量的溶酶体;相邻上皮细胞之间近顶端处有连接复合体;滤泡细胞基底部附着毛细血管壁。

实热造模组:上皮细胞略呈高柱状,上皮顶部胞质中有许多大小不一的卵圆形胶质颗粒;微绒毛发达、密集,有分枝;滤泡腔内胶质减少,并形成空泡;粗面内质网有不同程度扩张,有的融合成囊泡状,内含少许电子密度较低的絮状物;线粒体稍有增多,形状不规则。

虚热造模组:上皮细胞呈高柱状,上皮顶部胞质加宽,有许多较大的胶质颗粒;微绒毛发达;滤泡腔内有空泡状胶体颗粒;粗面内质网大都高度扩张,有的融合成不规则的囊泡,池腔内容物有的呈絮状,有的为均质状;高尔基体发达;核糖体丰富。

实热治疗组:上皮细胞较 I 组略高,但较 II 组略矮;粗面内质网仍有不同程度的扩张;线粒体略有肿胀;胶质颗粒减少,余结构与 I 组相近。

虚热治疗组:上皮细胞较 I 组略高,但较 II 组略矮;粗面内质网仍有轻度扩张,顶部胶质颗粒较多,余结构与 I 组相近。

3 讨论

甲状腺激素在维持机体生长发育、正常代谢及各种刺激的正常反应中起重要作用,甲状腺滤泡上皮细胞是甲状腺激素合成和分泌的场所^[5]。甲状腺滤泡上皮细胞的形态与激素的合成、分泌活动密切相关^[6]。

灌服健脾益气药、温热散寒药造成的实热证、虚热证模型大鼠对甲状腺激素的需要增加,甲状腺滤泡上皮细胞呈现增生性变化,上皮细胞呈高柱状,其中粗面内质网扩张和上皮细胞顶部胞质加宽,滤泡腔内空泡状胶质颗粒增多,标志着甲状腺滤泡合成和分泌甲状腺激素的功能活跃。甲状腺激素的主要作用是影响细胞核中基因的转录从而影响细胞中蛋白质的合成,其中包括氧化磷酸化所需要的酶^[7]。热证大鼠随着甲状腺激素分泌的增多,机体能量代谢增加,此时糖、脂肪、蛋白质的消耗虽然显著增加,消耗的氧也显著增加,但

是生物氧化产生的能量不能以 ATP 形式贮存,而是大部分以热能的形式散发^[7]。可见热证造模大鼠饮食、饮水量增大,体重降低,肛温升高,呼吸频率加快,与甲状腺功能改变引起的代谢加快、应激增强有关。

经过清热解毒中药(泻心汤)、滋阴清热中药(六味地黄汤)治疗后,虚热治疗组甲状腺激素水平显著下降,实热治疗组、虚热治疗组甲状腺滤泡上皮细胞结构与正常对照组接近,只有粗面内质网仍轻度扩张,说明中药治疗能够减少机体对甲状腺激素的需求量,纠正其过于旺盛的能量代谢。

虚热造模组大鼠甲状腺激素比实热造模组上升显著,电镜下的甲状腺超微结构改变也比实热造模组明显,表现出结构与功能的一致性。这种现象的出现可能是由于实热证时正邪相争,正气未伤,机体为了避免过度的代谢消耗所采取的一种自我保护反应。

甲状腺激素的合成及分泌由 TSH 和交感神经双重机制调节,TSH 的作用缓慢而时效长,神经的作用迅速而时效短^[8]。本实验实热证、虚热证造模大鼠血清 TSH 浓度均没有显著变化,分析其原因,可能是由于该实验热证模型造模时间不是很长(2 周),若适当延长造模时间,可能会出现 TSH 浓度的改变;也可能由于热证时的甲状腺激素水平主要由交感神经调节,这方面的研究有待进一步探讨。

热证状态是诱发部分亢进性疾病的内在原因^[9]。甲状腺功能亢进时血清中 rT_3 及 T_4 升高而 T_3 下降,本实验中阴虚内热证大鼠血清 T_3 及 T_4 水平升高, rT_3 水平呈下降趋势,说明甲状腺功能亢进与中医阴虚内热证的病理变化有相同之处,但不完全吻合。

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ABSTRACTS FROM ORIGINAL ARTICLES

Clinical Observation on Treatment of 240 Cases of Chronic Hepatitis of Syndromeless Differentiation Type with Self-Made Wen Gan Tablet

Wang Huameng

In order to probe into change of syndrome types and the necessity of treatment, 346 cases of chronic hepatitis of syndromeless differentiation type were randomly divided into two groups. 240 cases in the treatment group were treated with Wen Gan Tablet and 106 cases in the control group were treated with starch or did not receive any treatment. Within 6 months, it was investigated whether the patient in syndromeless differentiation type changed and the changed syndrome types. Results showed that the syndrome type was changed in 44 cases of the treatment group, and 62 cases in the control group, accounting for 18.33% and 58.49% respectively, with significant difference ($P < 0.01$). It is indicated that intensive treatment can be used for chronic hepatitis of syndromeless differentiation type to reduce attack of chronic hepatitis.

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Key words: hepatitis, virus, human/TCM therapy, @Wen Gan Tablet

(Original article on page 28)

Comparative Study on Ultramicrostructures and Functions of the Thyroid in Rats of Excess-Heat Type and Deficiency-Heat Type

Xu Zhiwei, Chen Qun, Liu Yamei, et al

Serum contents of triiodothyronine (T_3), tetraiodothyronine (T_4), reverse triiodothyronine (rT_3) and thyroid-stimulating hormone (TSH) and changes of structures of the thyroid gland in the rat of excess-heat syndrome and deficiency-heat syndrome were observed with radioimmunoassay and electron microscopic technique, respectively. The rats of excess-heat syndrome and deficiency-heat syndrome were established with administration of Chinese herbs. Results indicated that serum T_3 and T_4 levels increased and the rT_3 level tended to decrease in the deficiency-heat group, and thyroid follicle epithelial cell had more significant changes as compared with that of the excess-heat group. It is suggested that changes of hormones and ultra-microstructures of the thyroid can be served as an important basis for heat syndrome of deficiency type and excess type.

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Key words: heat of excess type/pathology, heat of deficiency type/pathology, thyroid gland, ultramicrostructure

(Original article on page 43)

Preliminary Study on TCM Crasiology in 10 Strains of Male Rats

Chen Xiaoye, Zhou Yongsheng, Zou Shijie, et al

Ten strains of male rats are commonly used for experimental study at present in Beijing. The rats' outward appearance, stool, picture of the tongue, body temperature, and wet weight of adrenal gland, thymus gland and spleen were observed, and the function of the hypothalamus-pituitary-thyroid axis was determined. Results showed that the constitution of Wistar grade I, II, III, and SD grade III were basically normal. SD grade I had the constitution of mild deficiency-cold, SD grade II had constitution of excess-heat, CD (SD) IGS grade III (small or big body weight) had constitution of mild deficiency. SHR grade I had constitution of mild deficiency-cold, SHR/sp grade I had obvious deficiency-cold constitution; There was a certain difference in TCM constitution among different strains, same strain with different microbic grades, pure line and inbred line, and different ages in rats. It is indicated that the study on TCM crasiology in experimental animals are not only necessary, and also feasibility.

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Key words: Crasiology/TCM

(Original article on page 45)

Effects of Jian Nao Yi Zhi Instant Granules on Contents of Monoamine Neurotransmitters and Substance P of Cerebral Cortex in Rats of Multi-Infarct Dementia

Mei Jianxun, Zhang Yunling, Zhang Boli, et al

In order to explore the mechanism of Jian Nao Yi Zhi Instant Granules for treatment of Multi-infarct dementia (MID), the contents of dopamine (DA), adrenaline, noradrenaline, 5-hydroxytryptamine (5-HT) and substance P in cerebral cortex were observed in the rat of MID with electrochemical detection of high pressure liquid chromatography and immunohistochemical method. Results indicated that the content of adrenaline in the rat of MID increased in the pre-operation Chinese herb group; 5-HT level decreased in the post-operation Chinese herb group and the post-operation western medicine (dihydroergotamine) group. Contents of substance P elevated in the three groups, with more increase in the pre-operation Chinese herb group. It is concluded that the prescription can regulate abnormal metabolism of monoamine neurotransmitters and substance P in the cerebral cortex after MID. This is possibly material base for improving memory.

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Key words: dementia, multi-infarct/TCM therapy cerebral cortex/metabolism @Jian Nao Yi Zhi Instant Granules

(Original article on page 48)