

冬虫夏草、大黄及肾大部切除大鼠血清 对肾小管上皮细胞生长的影响

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内容提要 本研究利用体外肾小管上皮细胞培养及氚标记的胸腺嘧啶核苷(^3H -TdR)掺入技术,观察了冬虫夏草(虫草)对细胞生长的影响,结果表明虫草可明显促进 ^3H -TdR向肾小管上皮细胞内掺入(虫草组 $11060.63 \pm 603.04\text{CPM}$,对照组 $8323.83 \pm 836.72\text{CPM}$, $P < 0.001$)。为此考虑虫草减轻肾小管损伤的作用之一在于促进细胞的修复。此外,我们还观察了大鼠5/6肾切除后血清及中药大黄对肾小管上皮细胞的影响,结果显示5/6肾切除后血清能明显刺激 ^3H -TdR掺入到上皮细胞内,而大黄则可抑制肾小管上皮细胞的生长。

关键词 冬虫夏草 大黄 肾大部切除 肾小管上皮细胞

许多研究证实庆大霉素造成肾损伤的同时存在着细胞修复过程,我们在大体及器官水平发现虫草可以减轻庆大霉素所致的小管损伤,考虑其作用之一在于促进受损细胞的修复。本研究利用体外肾小管上皮细胞培养技术及 ^3H -TdR掺入法,着重观察虫草与细胞增殖的关系。

此外许多研究表明肾小管的异常高代谢是慢性肾功能不全继续恶化的重要因素之一,探讨导致肾小管上皮细胞高代谢的因素是目前研究的重要课题。在此我们就5/6肾切除后大鼠血清及中药大黄对大鼠肾小管上皮细胞增殖的关系进行了研究。

材料与方 法

一、材料: (1)Wistar雄性大鼠,体重120~140g,本院动物室提供。(2)大黄 蒽醌片,中国药科大学提供。(3)冬虫夏草,市售四川产正品,用时煎沸浓缩至0.5g/ml。(4)RPMI1640培养液(GIBCO)。(5)胎牛血清,天津生化制品厂生产。(6)胰酶(DI-ECO)。(7)抗细胞角质素(Cytokeratin)抗体(DAKO)。(8) ^3H -TdR放射比度20Ci/mM中科院原子能研究所。(9)闪烁液: PPO 4.0g, POPOP 0.1g加甲苯1000ml溶解。

二、方 法

1.大鼠5/6肾切除术: 手术组取Wistar大鼠6只,以0.25%硫贲妥钠20mg/kg腹腔注射麻醉,腹正中切口长约2.0cm,暴露肾脏,剥离肾包膜,右肾结扎切除,左肾切除上、下两极后,用明胶海绵压迫止血,关闭腹腔。假手术组取同样大鼠6只,麻醉及切口同上,暴露,游离肾脏后关腹。两组大鼠术前、术后均

于室温下饲养,自由进食水,实验大鼠均于术后48小时于尾动脉无菌取血,分离血清, -20°C 保存。

2.含大黄代谢物实验血清的制备: 取大鼠5只,每只大鼠以大黄蒽醌每日0.3g/kg灌胃,7天后于尾动脉无菌取血,分离血清, -20°C 保存。

3.含虫草代谢物实验血清的制备: 取大鼠8只,均分为2组, A组以虫草煎剂1.0g/日灌胃, B组为对照组自由进食。于喂药第7日尾动脉无菌取血,分离血清,于 -20°C 保存。

另取大鼠2只,以同样剂量虫草灌胃,在灌胃前及喂药后1.5、3、4、5、6、7.5小时分别于尾动脉取血,分离血清, -20°C 保存,观察1次服药后虫草在体内浓度变化规律。

4.肾小管上皮细胞的培养与鉴定⁽¹⁾

(1)分离肾小管: Wistar雄性大鼠4只,体重60~80g,处死后无菌取肾,去包膜,分离肾皮质,剪碎后移入80目(孔径151 μ)不锈钢网筛,研磨并用生理盐水冲洗,于100目网筛上收集肾小管节段(过筛后小管纯度可达90%),将其置离心管内,用无血清RPMI1640培养液冲洗,1000r/min离心5min。

(2)肾小管节段的消化及培养: 上述小管节段离心后弃洗液,加0.2%胰酶1.5ml,置 37°C 水浴20min后,用等量胎牛血清中止消化,分装于预先用牛I型胶原处理的塑料培养瓶中,加入含7%胎牛血清的RPMI1640培养液至5ml。置孵育箱(37°C ; 5% CO_2 , 95% O_2)中培养。

(3)细胞免疫学鉴定: 用甲醇将细胞固定10min, 冲,再用磷酸盐缓冲液(PBS)洗3次,共10min。用抗Cytokeratin抗体行间接荧光染色,鉴定细胞种类。

5. 观察不同实验血清对 ^3H -TdR掺入细胞的影响: 将原代培养的小管上皮细胞(第6~7天)用0.05%胰酶、0.02%四乙酸二氨基乙酸钠(EDTA)在37°C水浴消化3分钟, 以10%胎牛血清(FCS)中止消化, 用培养液冲洗离心一次, 弃上清后加入无血清RPMI1640液制成细胞悬液, 计数后加至96孔培养板(100 μl /孔, 细胞数10 000个/孔)置37°C CO₂孵箱, 24小时后分别加入5/6肾切除后大鼠血清、含大黄及虫草代谢物血清和对照血清10 μl , 每份血清分设2~3个平行孔, 继续培养16小时, 每孔加0.5 μCi ^3H -TdR, 36小时后用多头细胞收集器收集细胞至49型滤纸, 烘干后放入闪烁瓶中, 每瓶加闪烁液5 ml, 用闪烁仪(LKB1215型)测定标本每分闪烁值(CPM值)。

6. 所有数据均经检验t处理。

结 果

一、肾小管上皮细胞的生长与鉴定

1. 肾小管上皮细胞的生长: 肾小管节段贴壁后约3天其外周开始长出细胞, 5~7天细胞呈指数生长, 7天左右生长良好, 可以融合, 铺满瓶底, 形态为多边形鹅卵石样, 见图1。

2. 细胞种类鉴定: 研究表明上皮细胞中间丝结构含有角蛋白成分, 培养第7天的细胞间接免疫荧光染色表明, 抗Cytokeratin抗体反应为阳性, 证明是上皮细胞。见图2。应用抗Cytokeratin抗体行间接免疫荧光染色细胞显阳性。

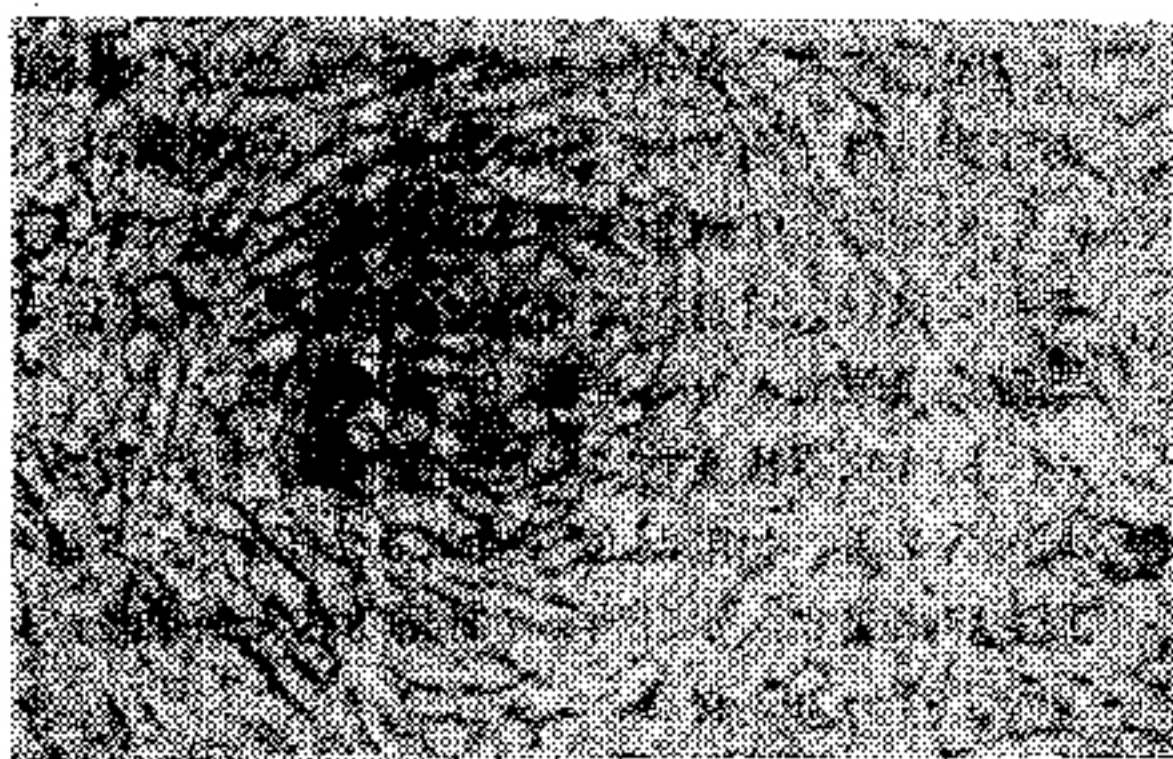


图1 原代培养第6天生长良好的肾小管上皮细胞 $\times 10$



图2 抗Cytokeratin抗体间接免疫荧光染色, 细胞呈阳性反应 $\times 40$

二、不同实验血清对 ^3H -TdR掺入细胞的影响

1. 5/6肾切除后48小时血清对细胞掺入 ^3H -TdR的影响: 5/6肾切除后48小时的血清可以明显促进 ^3H -TdR向细胞内掺入($3825.25 \pm 649.61\text{CPM}$), 与假手术组的血清比较($2806.33 \pm 688.99\text{CPM}$)相差非常显著, $P < 0.01$ (数据表示 $\bar{x} + S$, 下同)。

2. 大黄对肾小管上皮细胞掺入 ^3H -TdR的影响: 含大黄代谢物实验血清组细胞 ^3H -TdR掺入量为 $1788.87 \pm 295.89\text{CPM}$, 而正常对照组为 $2618.13 \pm 368.45\text{CPM}$, 两组比较相差显著。

3. 虫草对肾小管上皮细胞DNA合成的影响: 以 ^3H -TdR掺入作为反映细胞DNA合成的指标, 用虫草喂养大鼠的血清加入小管上皮细胞培养液, 发现肾小管上皮细胞对 ^3H -TdR的摄取明显增加, 测得CPM值在虫草组为 11060.63 ± 603.04 , 在对照组为 8323.83

± 836.72 , 前者比后者增加32.88%, $P < 0.001$ 。

一次服药后虫草在体内浓度变化规律, 如图3所示, 小管细胞对 ^3H -TdR摄入量于1.5小时及5小时有两次高峰, 6小时后血清中浓度下降。

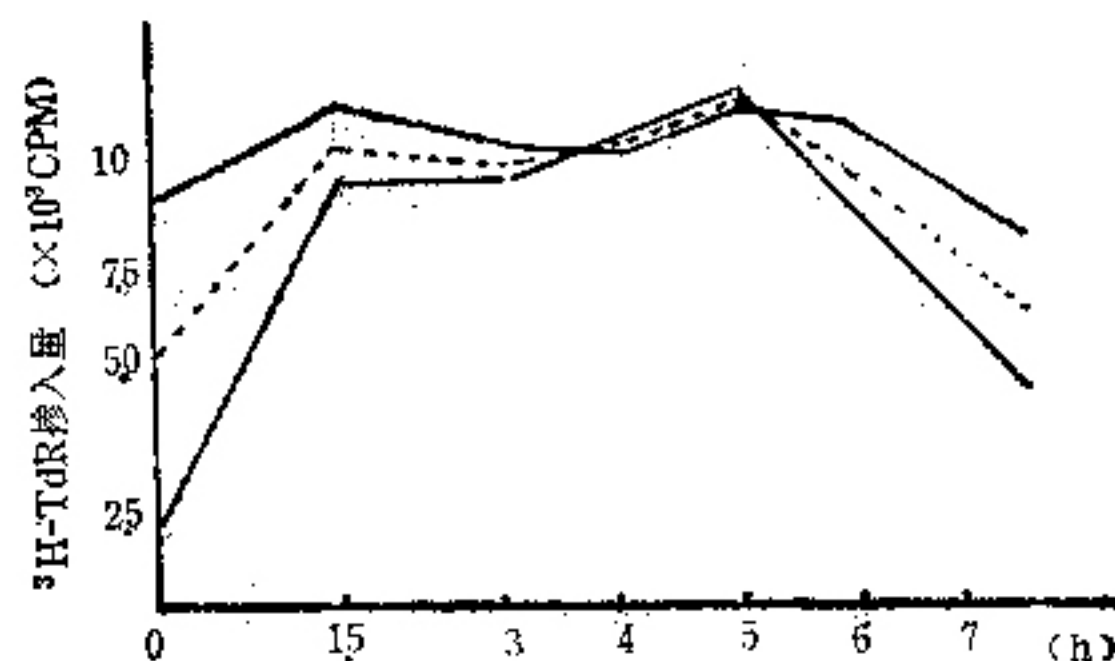


图3 一次服药后虫草在体内浓度变化规律

讨 论

一、5/6肾切除后血清对肾小管上皮细胞的影响：人们常用5/6肾切除大鼠动物模型观察肾衰进展。Brenner于1982年根据一系列实验研究资料提出了肾小球血流动力学变化学说⁽²⁾，受到人们的普遍重视，但其只强调了肾小球生理改变的重要性。1988年Schrier在此基础上提出了肾小管高代谢学说，指出大量肾单位减少后残余肾单位处于异常高代谢状态，其结果反而使肾小管细胞氧耗量增加，脂质过氧化物增多，细胞内碱化及酶活性异常等，从而进一步造成细胞损伤⁽³⁾。本实验证实大鼠5/6肾切除后血清确实存在一种迅速升高的活性因子（促肾因子），它对体外培养的肾小管上皮细胞有明显的刺激作用，导致细胞DNA合成的迅速增加。关于促肾因子活性的变化规律，蒋功伟等观察到5/6肾切除术后该因子活性迅速升高，第48小时达到高峰，以后迅速下降，第28天时已降至正常水平^(4,5)。这种因子的化学结构及产生部位目前还不很清楚，有研究表明可能存在于垂体⁽⁶⁾，正常机体促肾因子含量低且处于被抑制状态，当大部分肾切除或随着有效肾单位的减少，可能存在的抑制被解除，使促肾因子活性增加，从而使细胞处于高代谢状态。为此降低或抑制病理情况下出现的这种不利于机体的高代谢状态，对减少代谢产物致组织细胞的损伤及延缓慢性肾衰的进展是有益的。

二、大黄对肾小管上皮细胞生长有抑制作用：动物实验表明用大黄提取物治疗5/6肾切除大鼠，8个月后BUN及血肌酐上升速度明显减慢，离体灌注肾示氧耗量下降。本实验观察了大黄对原代培养的小管上皮细胞的影响，发现含有大黄代谢物的血清，能显著抑制肾小管上皮细胞对³H-TdR的摄取量，而且此种抑制并非大黄毒性的直接杀伤作用⁽⁷⁾。这说明大黄对细胞DNA的合成有抑制作用，对于减低慢性肾衰时肾小管的高代谢有一定的影响。这一结果为临床应用大黄治疗慢性肾衰提供了理论依据。

三、冬虫夏草对肾小管上皮细胞增长的影响：作者等曾利用大鼠腹腔注射庆大霉素，造成急性肾小管损伤，观察到用虫草治疗的实验大鼠血尿素氮及尿中NAG酶、溶菌酶均明显低于对照组，说明虫草可以减轻庆大霉素的肾脏损害⁽⁸⁾。另外虫草可使离体灌注肾的氧耗量增加。许多研究表明，在持续应用庆大霉素造成肾损伤的同时存在着修复过程⁽⁹⁾。Laurent和Tulkens能减轻大鼠应用小剂量庆大霉素(10mg/kg·

d) 4天后³H-TdR掺入到肾皮质DNA的量明显增加，认为肾皮质DNA合成的增加意味着庆大霉素使肾小管损伤后肾小管上皮细胞的修复⁽¹⁰⁾。本实验结果表明虫草可以明显促进³H-TdR向小管上皮细胞内掺入，为此我们考虑虫草减轻急性肾小管细胞损伤的作用之一在于它可促进受损细胞的修复。

在过去的十年中，人们对于急、慢性肾功能不全的研究已从整体动物、离体灌注肾、肾小管微灌注，深入到细胞、细胞器以致膜、受体，不断阐明各种因素对肾衰的影响，加深了人们对肾衰的认识。我们于国内较早成功的进行了大鼠原代肾小管上皮细胞的培养，并且观察了临床上对于肾衰有较好疗效的中草药在细胞水平对于肾小管的影响，这一实验结果为临床应用虫草及大黄治疗肾功能不全提供了一定的理论基础。

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Prevention and Treatment of Isoproterenol Induced Ventricular Fibrillation in Rats

by Aqueous Extract of *Salvia miltiorrhiza*

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Acute fatal ventricular fibrillation (VF) in male Sprague-Dawley rats was induced by subcutaneous injection of isoproterenol (1 mg or 5 mg/kg body weight) to two groups of rats of different body weights (525 ± 21 g or 387 ± 11 g) respectively. VF occurred in all control rats resulting in 96% death with only 4% spontaneously reverted and survived. Pretreatment of animals, with or without pentobarbital anaesthesia, with an aqueous extract of *Salvia miltiorrhiza* (SM-H, i. p., 5 g herb/kg body weight) significantly reduced J-point displacement and VF induced by isoproterenol. Survival rate was significantly raised compared with the control ($P < 0.05$). Immediate intravenous injection of SM-H (5 g herb/kg body weight) to poisoned rats which developed VF caused 71% of them to recover temporarily their sinus rhythm and significantly prolonged their survival time ($P < 0.05$).

Key Words *Salvia miltiorrhiza*, isoproterenol, ventricular fibrillation

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Effects of *Cordyceps sinensis*, Rhubarb and Serum Renotropin on Tubular Epithelial Cells Growth

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Primary cultured rat tubular epithelium was utilized to investigate the effect of *Cordyceps sinensis* (CS) on cellular proliferation and metabolism. Judging from incorporation rate of ^3H -TdR, it was found that the addition of serum containing CS metabolites into the culture media could promote the DNA synthesis of tubular cells profoundly ($P < 0.001$). In association with its beneficial effects on gentamycin nephrotoxicity in vivo study, it is indicated that CS could enhance the regeneration of injured tubular cells. In addition, sera obtained from 5/6 nephrectomized rats (5/6 NT) and rhubarb treated rats were studied to see their effects on tubular cells growth. The results showed that the serum of 5/6NT rats could promote the DNA synthesis of tubular epithelium, while the presence of experimental serum of rhubarb in culture media markedly inhibited the DNA synthesis of cells.

Key Words *Cordyceps sinensis*, rhubarb, nephrectomy, tubular epithelial cell

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The Experimental Study of The Heat and Cold Constitutional Types (II)

A Comparative Observation on the Heat and Cold Constitutional Type Rats on the DNA Replication Capacity after Ultraviolet Damage and the Proliferation Capacity of the Lymphocytes in vitro

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The cold-constitutional and the heat-constitutional type had selected in Wistar rats as the object of study. Using peripheral blood and spleen as materials by means of ultraviolet injury, isotope incorporation, cells incubation in vitro and liquid scintillation counting, the capacity of peripheral lymphocytes DNA replication after damage with ultraviolet radiation and the capacity of the spleen lymphocytes proliferation in vitro was observed. The results showed that the both capacities mentioned above were higher in the heat constitutional type rat than that in the cold type. It is suggested that the following conclusion in the Lingshu Jing(灵枢经) is correct: "the capacity of tissue repair is higher in the heat constitutional type than in the cold."

Key Words constitution, heat-constitutional type, cold-constitutional type, DNA replication synthesis, cells proliferation

(Original article on page 550)