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Effects of different types of operation on hepatic reserve function in rats with cirrhotic portal hypertension

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Abstract Objective: To investigate the effects of different types of operation on hepatic reserve function in cirrhotic portal hypertension. Methods: The Wistar rats with cirrhotic portal hypertension induced with carbon tetrachloride/ethanol were divided into four groups: 1) DSCS; 2) PAD; 3) MCS and 4) the control. The changes of hepatic reserve function were observed with oral glucose tolerance test (OGTT) and arterial ketone body ratio (KBR) determination. Results: 1) At the end the 1st week, blood glucose (BG) values of OGTT in the four groups were 11.2, 11.4, 13.0 and 13.5 and KBR were 0.340, 0.349, 0.326 and 0.335 respectively. 2) At the end of the 3rd and 5th week, BG in the first three groups were decreased to 62%, 63%, 93% and 59.2%, 62.9%, 92.6% of that in the control and KBR was increased to 139.4%, 144.5%, 100.1% and 171.6%, 189.8%, 97% to that in the control respectively. Conclusion: DSCS and PAD are better than MCS in protecting the hepatic reserve function in rats with cirrhotic portal hypertension.

Key words liver cirrhosis; hepatic reserve function; arterial ketone body ratio; rat

炎症诱导性表达载体的构建及白介素-10基因转染对烫伤内毒素血症小鼠保护作用的实验研究*

Construction of inflammation inducible expression vector and protection of experimential IL-10 gene transfection on endotoxemia and scald mouse

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失控性炎症细胞因子、过量的介质释放介导、推动系统性炎症反应(SIRS)、脓毒性反应及多器官功能衰竭,开发、寻求新的抗炎症因子策略以处理失控性炎症反应是当今烧伤、创伤及外科领域的迫切任务。白细胞介素-10(IL-10)又名"细胞因子合成抑制因子(CSIF)"广泛抑制多种炎症介质的合成提示 IL-10 可能用于感染性休克、脓毒症及 SIRS的抗介质综合治疗。

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H+ concentration, damage index and NO concentration in the gastric mucous membrane were determined. **Results:** In the experimental group, the potential differences, blood flow and NO concentration in the gastric mucous membrane were significantly decreased while the H+ concentration and damage index in it significantly increased. Administration of collagen solution to the rats in the experimental group beforehand resulted in the restoration of the above indices ($P < 0.01$ or 0.05). **Conclusion:** The ischemia and hypoxia caused by insufficient blood perfusion is one of the important factors in the pathogenesis of injury of the gastric mucous membrane induced with bound and frozen irritation. Administration of collagen solution to the rats can prevent the direct injury of various factors to the gastric mucous membrane through maintaining the intactness of the gastric mucous membrane, buffering gastric acid, improving coacervation of the platelets and increasing the cellular DNA content.

Key words irritable injury of the gastric mucous membrane; collagen; prevention; rat

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本研究发展新的抗介质基因治疗策略通过应用急性相蛋白启动子(血清淀粉样蛋白 A)SAA3启动子对治疗基因(白介素-10)表达进行调控,调控 IL-10在局部组织表达,使其表达有可控制性,呈自适应表达。IL-10作为目的基因可广泛地下调单核/巨噬细胞系统活化及广泛控制多种炎性介质产生,而又不致过度表达抑制免疫功能及防御能力,使炎症反应控制在适度水平。观察 hIL10基因转染对 LPS休克模型的保护作用。探索供细胞因子基因治疗中目的基因受机体病理信号调控的方法——炎症诱导性启动子指导的目的基因表达的可能性及利用基因治疗方法探索对失控性炎症信号级联途径的中断及阻滞作用。

主要的实验结果及结论如下:

(1)血清淀粉样蛋白 SAA3增强子/启动子表达体系其体外、内的诱导表达活性分析。脂质体包裹的 pSAA3CAT转染的肝细胞株 SMMC7721与单核细胞 U937有低水平报告基因 CAT的表达,rIL-6+ rIL-1联合活化肝细胞株 SMMC7721可迅速诱导报告基因表达,6 h开始启动,24 h达峰值,72 h时已下降;LPS可启动转染单核细胞 U937细胞报告基因的表达;rIL-6+ rIL-1可剂量依赖性增加转染肝细胞 SMMC7721及单核细胞 U937细胞的 CAT表达。

腹腔注射脂质体包裹 pSAA3CAT体内基因转染,内毒素攻击可诱导报告基因主要在肝脏表达,LPS攻击转染鼠肝脏 CAT 12 h即可检出,24 h达峰值,120 h仍可检出;肝 CAT表达水平与 LPS剂量有剂量一效应关系。

(2) IL-10诱导性表达载体构建及体内、外表达分析。利用 SAA3启动子5′区(-306 bp+47 bp)片段连接 hIL10基因构建真核表达载体,pSAA3-hIL10成功表达于腹腔巨噬细胞及肝/kupffer细胞,hIL10特异性引物 RT-PCR分析、Western blot分析证实该表达载体成功转移并转录出完整的 mRNA分子并翻译出 hIL10分子。

RT-PCR分析证实转染 pSAA3 hIL10的腹腔巨噬细胞,hIL10表达有炎症诱导性。单剂量 LPS诱导 hIL10表达持续时间为120 h,LPS活化后 12 h即有 hIL10蛋白表达,峰值在 24~48 h,96 h hIL10已显著下降;同时可观察到 LPS活化转基因 MΦ细胞 TNF-α产生显著减少(37.6%),显著下调 IL-6的产生(42.8%);在肝/kupffer细胞混合培养系统,IL-10转基因下调混合培养系统 TNF-α产生(57.7%),显著抑制 IL-6的产生(36.8%);减轻 LPS活化后肝细胞的损伤,降低 AST、ALT、LDH的升高,$P < 0.05$

在小鼠内毒素血症模型,腹腔注射 pSAA3 hIL10转基因,RT-PCR分析证明脂质体包裹 DNA复合物可有效地体内转基因,LPS可诱导转录出完整的 hIL10 mRNA分子并有效地翻译出 hIL10蛋白。LPS显著诱导外源 hIL10在小鼠肝脏表达(mRNA),一次剂量的 LPS攻击后5 d内小鼠肝脏均有 hIL10表达;烫伤可显著延长 hIL10的表达(10 d内可检出);基因转染处理小鼠在 LPS攻击后小鼠血清 TNF-α下降44.75%~53.66%,血清 IL-6下降38.02%~38.1%,$P < 0.05$

(3) IL-10基因转染对烫伤小鼠肝脏局部炎性分子表达的抑制作用。原位杂交显示 hIL10基因转染预处理显著减少烫伤小鼠肝脏 TNF-α表达,减少肝脏 iNOS的表达,抑制肝脏粘附分子 ICAM-1 mRNA表达。IL-10基因转染预处理烫伤组形态学观察显示肝细胞水肿,肝细胞局灶性坏死减轻;电镜观察肝细胞、Kupffer细胞、内皮细胞超微结构病变减轻。基因转染处理抑制小鼠肝脏局部炎症介质分子的表达对肝细胞损伤,肝组织形态学的改变有一定的意义。

(4) IL-10基因转染提高内毒素血症小鼠的存活率。0.5~1.0 μg重组人 IL-10可显著减少 LPS(350 μg/mouse)攻击小鼠的死亡率;hIL10基因转染显著提高 LPS攻击小鼠的存活率,基因转染预处理组内毒素血症小鼠的存活率为42.6%,而对照组的存活率为26.8%(72 h),$P < 0.05$

关键词 白细胞介素-10;SAA3启动子;报告基因;基因治疗;巨噬细胞;Kupffer细胞;内毒素;烫伤;RT-PCR;TNF-α;IL-6;iNOS;ICAM-1

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