热毒清抗内毒素 DIC 家兔肝细胞和线粒体过氧化损伤的实验研究

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内容提要　本实验采用内毒素所致家兔 DIC 模型，检测肝组织及线粒体脂质过氧化物(LPO)、超氧化物歧化酶(SOD)及谷胱甘肽过氧化物酶(GSH-Px)。结果表明模型组 LPO 明显升高($P<0.01$)，SOD及GSH-Px活性明显下降($P<0.01$)；而热毒清治疗组 LPO 增高不明显($P<0.05$)，SOD及GSH-Px活性亦不下降($P>0.05$)。提示：在内毒素 DIC 时，肝细胞及线粒体对自由基清除功能下降、脂质过氧化损伤较重；而热毒清制剂在内毒素 DIC 时，增强了对自由基的清除功能，防止了过氧化损伤，对保护肝细胞和线粒体起到了良好作用。

关键词　内毒素　播散性血管内凝血　脂质过氧化物　超氧化物歧化酶　谷胱甘肽过氧化物酶　热毒清

近年来，越来越多的研究表明，具有高度活性的氧自由基 (OFRs) 是导致许多疾病病理过程中组织细胞损伤的重要因素。OFRs 在内毒素引起休克病理过程中起着重要作用[1~3]。但对 OFRs 在内毒素引起播散性血管内凝血(DIC) 病理过程中的作用报道甚少，对OFRs 在内毒素 DIC 时对肝细胞和线粒体损伤及其防治，还缺乏深入研究。本实验采用内毒素诱发家兔全身性Shwartzman反应，塑造DIC模型。测定肝匀浆上清液及其线粒体悬液中自由基脂质过氧化反应终末代谢产物 (MDA) 的含量，内源性谷胱甘肽过氧化物酶(GSH-Px)及超氧化物歧化酶(SOD) 活性的变化，旨在探讨OFRs 在内毒素 DIC 时对肝细胞、线粒体的损伤作用及热毒清的防治效果。

材料和方法

一、动物模型的制作和分组：体重为1.8～2.5kg的日本大耳白兔48只，雌雄不拘，随机分为四组，每组12只。除正常对照组(正常组)不作任何处理外，其余动物均间隔 24h 两次静脉注射(下简称静注)大肠杆菌 $O_{111}B_4$ 内毒素(上海生物制品研究所)，制成 DIC 模型。分为生理盐水组(模型组)：第 1 次注射内毒素后立即静注生理盐水 7ml/kg，以后每12h静注生理盐水 7 ml/kg，共 3 次；热毒清组：每次静注 100%的热毒清注射液（由金银花、蒲公英、大青叶及鱼腥草组成，宜昌民康制药厂产品）7 ml/kg；维生素 E 组(VE组)：每次大腿肌肉注射 VE 20mg/kg，后两组用药次数、时间均同模型组。

二、检测指标：动物于第 2 次内毒素注射后 10h 放血处死，立即取右叶肝组织匀浆后再按低温差速离心方法分离肝线粒体及肝匀浆上清液，作以下测定。

1. 肝组织及线粒体脂质过氧化物(LPO)测定：按 Ohkawa法[4]，结果以 nmol MDA/mg protein 表示。蛋白质定量按Lowry法[5]。

2. 肝组织及线粒体 GSH-Px测定：按二硫代二硝基苯甲酸(DTNB)法[6]，结果以nmd/min·mg protein 表示。

3. 肝组织SOD测定：按邻苯三酚自氧化法，结果以u/mg protein表示。

实验数据按单因素方差分析进行显著性检验，部分数进行相关分析。

结　果

一、各组肝组织及线粒体LPO含量：模型组肝组织及线粒体 LPO 均明显增高，VE 组仅轻度增高，而热毒清组增高不明显(见附表)。

二、各组肝组织及线粒体GSH-Px 活性：模型组均明显降低而VE组及热毒清组均不降低(见附表)。

三、各组肝组织 SOD 活性：模型组明显下降，而VE组及热毒清组与正常组接近(见附表)。

四、GSH-Px、SOD与 LPO 间的关系：模型组、热毒清组及VE 组肝组织，线粒体 GSH-Px与 LPO 呈负相关($P<0.05$或<0.01)；热毒清组SOD也与 LPO 呈负相关($P<0.01$)，其余各组无相关性 ($P<0.05$，图均略)。

附表　肝组织、线粒体 LPO、SOD 及 GSH-PX 测定结果　($\bar{X}\pm S_{\bar{x}}$)

组　别	兔数	肝　组　织			线　粒　体	
		LPO	SOD	GSH-Px	LPO	GSH-Px
模　型	12	1.538±0.252	13.53±3.43	99.25±20.53	0.955±0.125	20.74±3.68
热毒清	12	1.126±0.190**	20.29±3.67**	135.51±15.26**	0.702±0.093**	35.49±5.22**
VE	12	1.324±0.213*△	17.79±2.66**	132.11±13.36**	0.774±0.146**△	35.60±5.43**
正　常	12	1.137±0.164**	19.53±3.42**	135.18±16.22**	0.641±0.097**	32.30±3.11**

注：1. LPO、SOD及GSH-Px单位分别为：nmol MDA/mg protein、u/mg protein及nmol/min·mg protein。2. 与模型组比较，*$P<0.05$，**$P<0.01$；与正常组比较，△$P<0.05$

讨　论

一、OFRs与内毒素DIC肝细胞、线粒体损伤的关系：正常情况下，体内产生少量的 OFRs 可被体内的 OFRs 清除系统如SOD、GSH-Px和过氧化氢酶等迅速清除，不致于堆积过多引起组织细胞的损伤。但在病理情况下，机体内OFRs清除功能下降而导致 OFRs 增多，则可造成组织细胞的严重损伤。本实验结果表明：模型组肝组织及线粒体 LPO 明显增高；肝组织 SOD及肝组织、线粒体GSH-Px 活性均明显下降，且 LPO与GSH-Px 两者呈负相关。而用自由基清除剂的 VE组LPO明显低于模型组，其SOD及GSH-Px活性亦明显高于模型组。结果提示：在内毒素DIC 病理过程中，肝细胞及线粒体内OFRs含量增多，其增多与 GSH-Px、SOD 活性下降有关；增多的 OFRs 导致了肝细胞和线粒体的过氧化损伤。内毒素DIC时肝细胞及线粒体等细胞器损伤已被证实[7]，尽管内毒素DIC时有许多因素可导致肝细胞及线粒体等细胞器的损伤，但 OFRs 引起的脂质过氧化可能是导致其损伤的重要因素之一。

二、热毒清对内毒素DIC 时肝细胞和线粒体脂质过氧化损伤的防治作用：热毒清临床用于治疗多种急性感染性疾病，疗效满意。实验研究证明热毒清具有抑菌、抗炎、增强免疫功能，拮抗内毒素所致DIC 生物效应，保护溶酶体和线粒体等功能[7]。

本实验结果：热毒清组能完全抑制内毒素DIC 家兔肝组织及线粒体LPO的增高，并能显著提高肝组织 SOD及肝组织、线粒体 GSH-Px 活性；且 GSH-Px、SOD与 LPO 呈显负相关，酶活性越高则 LPO 含量越低；本研究所曾证明热毒清组于家兔内毒素 DIC 时肝细胞、溶酶体及线粒体受损不明显[7]。提示：热毒清能通过提高OFRs 清除酶——SOD、GSH-Px 活 性，加速对内毒素DIC病理过程中产生的OFRs的清除；对内毒素DIC 时肝细胞及线粒体脂质过氧化损伤具有一定的防治作用。

内毒素DIC 的各种表现常见于中医温热病的严重阶段，较为难治。因此，从中西医结合观点来看，OFRs 亦可视为"热毒"的一种，清热解毒的现代涵义也应包括对自由基的清除作用。从传统医药中寻找有效的抗氧化药物，可望为临床上防治过氧化损伤性疾病开辟新的途径。

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by RIA and the parameters of triiodothyronine receptors in rat hepatic cell nucleus by radio-ligand binding assay: Maximal binding capacity (Bmax) and Dissociation constant (Kd). It is found that (1) Yin-tonics can lower serum thyroid hormone levels and Bmax of hepatic nuclear T_3R of hyperthyroxinemia rat from 167.14±25.62 fmol/100 μg DNA to 98.98±15.24 fmol/100 μg DNA, $P<0.001$. (2) Both Yang-tonics I and II can raise serum thyroid hormone levels of hypothyroxinemia rats, but not Bmax of hepatic nuclear T_3R. Yang-tonics I even lowers Bmax. All the Chinese herbs have no effect on the Kd of rat hepatic nuclear T_3R. The results may have some value in studying the effects of Chinese medical drugs.

Key Words hepatic cell nucleus, thyroid hormone receptor, Yin tonics, Yang tonics

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Study of Da Cheng Qi Tang(大承气汤) on ^{45}Ca Content of the Isolated Colon Smooth Muscle from Experimental Colon Obstruction Rats

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Da Cheng Qi Tang (DCQT) is a classical prescription of Chinese medicine for treatment of acute intestinal obstruction. In this paper, the isolated colon smooth muscle from normal and experimental colon obstruction (CO) rats were used to study the effect of DCQT on ^{45}Ca content. The results showed that the ^{45}Ca content on isolated colon smooth muscle (μm/g wet tissue, $\bar{x}\pm S$) was 0.043 ±0.009 in the normal and 0.057±0.012 in those treated by DCQT respectively. The content of ^{45}Ca in CO was higher than the normal, DCQT can reduce the content of ^{45}Ca in CO. It is known that the higher level of intracellular Ca^{++} is related to the formation and the development of acute intestinal obstruction. The inhibitory effect of DCQT on ^{45}Ca content may play an important role in the treatment of acute colon obstruction.

Key Words Da Cheng Qi Tang, colon smooth muscle, colon obstruction, ^{45}Ca content

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Preventive Effect of Re Du Qing(热毒清)on Hepatocytes and Mitochondria Damaged by Lipid Peroxidation in Experimental Rabbits with Endotoxin-Induced DIC

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In this study, the general Shwartzman reaction of rabbits induced by Escherichia Coli endotoxin was made as DIC models. The experiments showed that the levels of lipid peroxide (LPO) in hepatic tissue and mitochondria in the model group were increased significantly compared with the control group ($P<0.01$), while superoxide dismutase (SOD) activity in hepatic tissue and glutathione peroxidase (GSH-Px) activity in hepatic tissue and mitochondria were decreased significantly ($P<0.01$). The levels of LPO in hepatic tissue and mitochondria in Re Du Qing (RDQ) group and vitamin E (VE) group were decreased significantly ($P<0.01$ and $P<0.05$ respectively) compared with the model group. The levels of LPO in the RDQ group did not differ from the control group ($P>0.05$), but the levels of LPO in the VE group were still higher than those in the control group significantly ($P<0.05$). The SOD activity in hepatic tissue and GSH-Px activity in hepatic tissue and mitochondria in both RDQ group and VE group were also significantly higher than those in the model group ($P<0.01$). These data suggest that the levels of oxygen free radicals were increased in hepatocytes and mitochondria. This is related to the decreased activities of SOD and GSH-Px in the course of pathogenesis of endotoxin-induced DIC. This study indicates that lipid peroxidation might be one of the important mechanisms resulting in hepatocellular and mitochondria from oxidative damage.

Key Words endotoxin, disseminated intravascular coagulation, lipid peroxide, superoxide dismutase, glutathione peroxidase, Re Du Qing

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