·临床研究·

辛伐他汀预处理对Pringle手法肝细胞癌切除术后肝功能的影响及机制

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[摘　要]　**目的**:探讨辛伐他汀(simvastatin)预处理对Pringle手法肝细胞癌切除术后肝功能的影响及其机制。**方法**:73例肝细胞癌患者随机分为对照组(n=35)和Simvastatin组(simvastatin预处理,n=38,患者术前3 d按20 mg/d口服simvastatin)。分别于手术开腹后和关腹前收集部分肝组织作为标本,于术前和术后第1天采集患者血清。测定血清丙氨酸氨基转移酶(alanine aminotransferase,ALT)、天门冬氨酸氨基转移酶(aspartate aminotransferase,AST)、总胆红素(total bilirubin,TBIL)和白蛋白(albumin,ALB)以评估肝细胞损伤;逆转录聚合酶链反应(RT-PCR)分析炎症因子白细胞介素(interleukin,IL)-1、IL-6、诱导型一氧化氮合酶(inducible nitric oxide synthase,INOS)及肿瘤坏死因子(tumor necrosis factor,TNF)-α水平。试剂盒检测血清丙二醛(malondialdehyde,MDA)和超氧化物歧化酶(superoxide dismutase,SOD)水平;Western blot检测内质网应激蛋白(C/EBP-homologous protein,CHOP)水平。**结果**:Simvastatin组术后第1天血清ALT、AST和TBIL水平显著低于对照组(P < 0.05);RT-PCR提示Simvastatin组IL-1、IL-6、INOS及TNF-α水平较对照组明显降低(P < 0.05)。Simvastatin组血清MDA水平明显低于对照组(P < 0.05);SOD水平显著高于对照组(P < 0.05)。Western blot显示Simvastatin组CHOP表达水平较对照组减少。**结论**:对于肝细胞癌患者,辛伐他汀预处理可有效缓解Pringle手法肝部分切除术后的肝功能损伤,机制可能与抑制氧化应激和内质网应激水平有关。

[关键词]　辛伐他汀;Pringle手法;炎症反应;氧化应激反应;内质网应激

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Effects and mechanisms of simvastatin pretreatment on liver function in hepatocellular carcinoma(HCC) patients undergoing Pringle's hepatectomy

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[**Abstract**]　**Objective**: This study aims to investigate effects and mechanisms of simvastatin pretreatment on liver function in HCC patients undergoing Pringle's hepatectomy. **Methods**: Total 73 HCC patients were randomly divided into control group (n=35) and simvastatin pretreatment group (patients were given oral simvastatin 20 mg/d, n=38). Pre-hepatectomy hepatic biopsies were harvested after laparotomy (prior to hepatic portal occlusion), and post-hepatectomy hepatic biopsies were obtained after reperfusion (prior to abdominal closure). Serum was collected before operation (Pre-Op) and on the first day after operation (POD-1). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL) and albumin (ALB) were measured to evaluate liver injury, and the levels of inflammatory cytokines interleukin (IL)-1, IL-6, inducible nitric oxide synthase (INOS) and tumor necrosis factor (TNF)-α were analyzed by reverse transcriptase polymerase chain reaction (RT-PCR). The levels of serum malondialdehyde (MDA) and superoxide dismutase (SOD) were detected by kits according to the manufacturer's directions. Endoplasmic reticulum stress-related protein C/EBP-homologous protein (CHOP) was analyzed by Western blot. **Results**: The serum levels of ALT, AST and TBIL in POD-1 were significantly lower in simvastatin group than those in control group (P < 0.05), and RT-PCR results showed the

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levels of IL-1, IL-6, INOS and TNF-α, were significantly lower in simvastatin group than those in control group ($P < 0.05$). Furthermore, serum MDA was lower in simvastatin group than that in control group ($P < 0.05$); SOD in simvastatin group was significantly higher than that in control group ($P < 0.05$). Western blot results displayed that the expression of CHOP was lower in simvastatin group than in control group. **Conclusion**: Simvastatin pretreatment for HCC patient effectively alleviates liver injury after Pringle's hepatectomy, and its mechanism could be related to the inhibition of oxidative stress and endoplasmic reticulum stress.

[**Key words**] simvastatin; Pringle maneuver; inflammatory reaction; oxidative stress reaction; endoplasmic reticulum stress

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肝门部阻断(Pringle)手法和全肝血流阻断法是肝部分切除或肝移植中常用的肝血流阻断方法。长时间血流阻断将引起严重的肝脏缺血再灌注损伤(ischemia reperfusion injury, IRI),可导致进行性肝细胞损伤,甚至患者死亡,被认为是肝部分切除术或肝移植术后早期肝功能不全或肝功能衰竭的主要因素[1-4]。因而,探寻肝脏IRI的有效防治手段对改善肝切除和肝移植手术预后至关重要。

既往研究表明他汀类药物可改善循环死亡后供者肝脏和肾脏的IRI[5-6]。在临床应用中,他汀类药物是HMG-CoA还原酶抑制剂,具有抗血栓和抗炎作用。此外,他汀类药物通过调节内皮一氧化氮、血红素加氧酶-1、内皮素-1、微血管稳定性和氧化应激等方式,具有保护血管和心脏的作用,可作为心血管疾病初级和二级预防的降胆固醇药物[7-10]。在心脏移植中,给器官供者应用辛伐他汀(simvastatin)可有效减少心脏移植物的血管病变,抑制同种异体心脏移植IRI,提高患者存活率[11]。然而,关于辛伐他汀在临床肝切除肝脏IRI中的作用及其机制尚未完全明确。本研究收集临床肝细胞癌患者Pringle手法肝部分切除的肝组织和血清,分析辛伐他汀组和对照组肝损伤、炎症因子及内质网应激水平,旨在明确辛伐他汀预处理对Pringle手法肝部分切除肝脏IRI的影响,并探讨其可能机制。

1 对象和方法

1.1 对象

这是一项前瞻性、单盲、随机研究,并经医院伦理委员会同意。本研究于2018年11月—2020年2月在南京医科大学第一附属医院肝胆中心完成。参与研究的肝细胞癌患者均在血流阻断的情况下进行肝切除术,每个参与者都签署了知情同意书。纳入标准:①Child-Pugh分级为A/B级;②手术中采用Pringle手法阻断肝门血流。排除标准:①年龄<18岁和>75岁的患者;②或有下列情形之一(完全失明和失聪;意识不清;阿尔茨海默病;神经性厌食症;酒精性精神错乱;癫痫;精神分裂症;麻痹性疾病;严重慢性感染;广泛转移肿瘤;艾滋病;胃溃疡出血;妊娠和哺乳;虚弱;营养不良;1型糖尿病;需要持续胰岛素注射的2型糖尿病;心脏衰竭;心律失常;尿毒症;继发性高血压);③术中实施肝动脉结扎者。所有患者随机分为对照组(n=35,术前禁食6 h)和Simvastatin组(n=38,患者术前3 d按20 mg/d口服辛伐他汀,术前禁食6 h)。分别于开腹和关腹前采集部分肝组织,术前和术后第1天采集患者血清。两组患者基本资料差异无统计学意义(P均>0.05),研究结果具有可比性(表1)。

辛伐他汀(MedChem Express公司,美国);兔抗人CHOP单克隆抗体,羊抗兔二抗(Cell Signaling Technology公司,美国);HE染色试剂、BCA蛋白浓度试剂、十二烷基硫酸钠-聚丙烯酰胺凝胶电泳试剂盒(杭州碧云天生物技术公司);TRIzol试剂(Invitrogen公司,美国);RT-PCR试剂盒(TaKaRa公司,日本);MDA及SOD试剂盒(中国江城生物技术公司)。低温离心机(Eppendorf公司,德国);Western blot转膜仪和凝胶成像分析系统(Bio-Rad公司,美国);RT-PCR仪器(Applied Biosystems公司,美国);RT- PCR系统(Thermo Forma公司,美国)。

1.2 方法

1.2.1 Pringle手法

间歇性使用Pringle手法已被证实是一种安全有效的措施,可用于减少肝脏部分切除的术中出血。在本中心,通常采用8Fr橡皮管绕过肝十二指肠韧带,在准备切肝前,将橡皮管收紧以阻断肝门血流。单次肝脏热缺血时间为10~15 min,复流时间为5 min,再阻断10~15 min,以此循环。

1.2.2 肝功能检测

所有血样均以3 000 r/min离心15 min后收集上清送至检验科检测血清丙氨酸氨基转移酶(alanine aminotransferase, ALT)、血清天门冬氨酸氨基转移酶

(aspartate aminotransferase,AST)、血清总胆红素(total bilirubin,TBIL)和血清白蛋白(albumin,ALB)水平。

1.2.3　Western blot分析

根据说明配置蛋白裂解液,加入研磨后的新鲜肝脏组织并置于冰上。使用涡旋仪每隔5 min震荡20 s,共6次。将EP管放入4 ℃离心机中14 000 r/min离心15 min后,将上清液移入新的EP管中,通过BCA法测蛋白质浓度。根据不同的蛋白质浓度计算上样量,随后电泳,转膜,封闭,一抗孵育过夜,二抗孵育2 h。根据说明配置显影液,显影并拍照记录。

1.2.4　RT-PCR实验

称取0.5 g肝组织,于液氮中研磨成粉状,TRIzol法提取总RNA,紫外分光光度计测量浓度,选择甘油醛-3-磷酸脱氢酶(glyceraldehyde-3-phosphate dehydrogenase,GAPDH)为内参,$2^{-\Delta\Delta}$CT法计算炎症因子mRNA相对表达量。引物设计如下:TNF-α上游为5′-CTGAGTTGACTCCTACTGTGGA-3′,下游为5′-TCTTCCCAGGGTCGATAAAGT-3′;IL-1上游为5′-GCAACTGTTCCTGAACTCAACT-3′,下游为5′-ATCTTTTGGGGTCCGTCAACT-3′;iNOS上游为5′-CCCTCACACTCAGATCATCTTCT-3′,下游为5′-GCTACGACGTGGGCTACAG-3′;IL-6上游为5′-TAGTCCTTCCTACCCCAATTTC-3′,下游为5′-TTGGTCCTTAGCCACTCCTTC-3′;GAPDH上游为5′-GGAGCGAGATCCCTCCAAAAT-3′,下游为5′-GGCTGTTGTCATACTTCTCATGG-3′。

1.2.5　血清MDA和SOD检测

按照试剂盒(武汉江城生物技术公司)使用说明书操作,结果以μmol/L表示。

1.3　统计学方法

统计分析均使用SPSS16.0统计软件。定量资料值以均值±标准差($\bar{x} \pm s$)表示。两组计量资料差异的比较采用t检验。$P<0.05$为差异有统计学意义。

2　结　果

2.1　患者一般资料和术中情况比较

两组患者一般资料:性别、年龄、最大肿瘤直径、凝血功能、Child-Pugh分级比较差异无统计学意义($P>0.05$)。两组患者术中资料:手术方式、肝门阻断时间、术中出血量、手术时间比较差异无统计学意义($P>0.05$,表1)。

表1　对照组和Simvastatin组患者一般资料和术中情况比较

Table 1　Comparison of demographic data and intraoperative condition between control group and Simvastatin group

项目	对照组(n=35)	Simvastatin组(n=38)	检验值	P值
性别(例)			χ^2=0.012	0.911
男	28	30		
女	7	7		
年龄(岁)	54.32 ± 7.29	56.72 ± 9.10	t=1.427	0.178
最大肿瘤直径(cm)	5.13 ± 3.10	5.64 ± 2.68	t=1.055	0.270
术前凝血功能				
PT(s)	12.30 ± 1.07	11.80 ± 1.25	t=0.961	0.316
APTT(s)	30.22 ± 4.01	31.70 ± 3.57	t=0.823	0.372
INR	2.71 ± 0.38	2.85 ± 0.28	t=0.369	0.718
Chikl-Pugh分级(例)			χ^2=0.892	0.344
A	34	35		
B	1	3		
手术方式(例)			χ^2=1.572	0.456
局部切除	20	27		
左半肝切除	9	7		
右半肝切除	6	4		
肝门阻断时间(min)	23.86 ± 1.33	26.14 ± 2.07	t=1.581	0.189
术中出血量(mL)	278.61 ± 81.53	251.66 ± 73.11	t=2.213	0.094
肿瘤TNM分期(例)			χ^2=0.226	0.634
Ⅰ+Ⅱ	29	33		
Ⅲ+Ⅳ	6	5		
手术时间(min)	138.75 ± 9.28	129.43 ± 11.02	t=0.441	0.667

2.2　患者术后肝功能比较

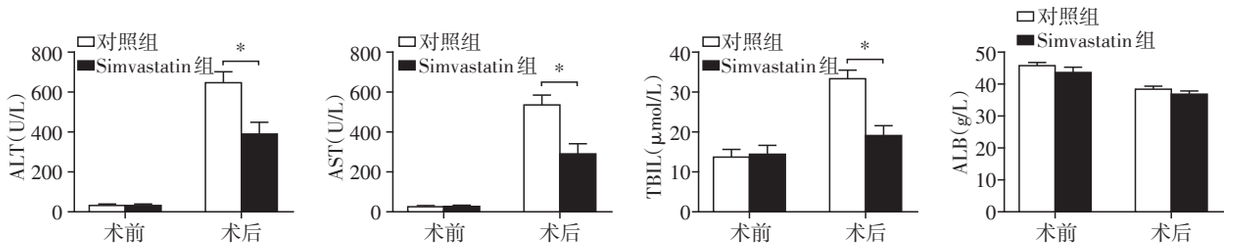
术后第1天时Simvastatin组较对照组的ALT[(389.4±80.7) U/L *vs.* (612.7±93.4) U/L]、AST[(293.4±87.3) U/L *vs.* (577.2±79.5) U/L]及[TBIL (18.7±2.6) μmol/L *vs.* (32.6±3.1) μmol/L]显著降低($P<0.05$，图1)。术前Simvastatin组与对照组上述指标比较差异无统计学意义($P>0.05$)。Simvastatin组ALB水平和对照组比较差异无统计学意义($P>0.05$)。

2.3　患者炎症因子表达水平比较

术后第1天，Simvastatin组与对照组比较，肝组织IL-1[(3.80±0.20) *vs.* (5.22±0.33)]、IL-6[(4.73±0.37) *vs.* (5.70±0.41)]、INOS[(3.78±0.30) *vs.* (6.21±0.27)]及TNF-α[(3.89±0.21) *vs.* (8.45±0.43)]mRNA水平显著降低($P<0.05$，$P<0.01$，图2)。两组术前以上炎症因子mRNA表达水平比较，差异无统计学意义($P>0.05$)。

2.4　患者术后血清MDA和SOD水平比较

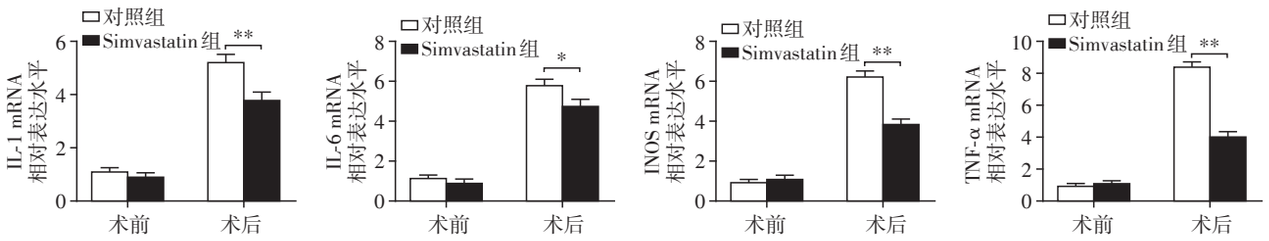
Simvastatin组术后第1天血清MDA[(7.16±0.78) μmol/L]比对照组[(14.90±1.02) μmol/L]低，血清SOD[(113.56±2.34) μmol/L]比对照组[(82.33±2.75) μmol/L]显著增加(图3)。术前两组MDA、SOD水平比较，差异无统计学意义($P>0.05$，图3)。



两组比较，*$P<0.05$。

图1　对照组和Simvastatin组患者术后肝功能比较

Figure 1　Comparison of postoperative liver function between control group and Simvastatin group



两组比较，*$P<0.05$，**$P<0.01$。

图2　对照组和Simvastatin组患者肝组织中炎症因子mRNA表达水平比较

Figure 2　Comparison of mRNA expression of inflammatory cytokines in liver tissues of patients between control group and Simvastatin group

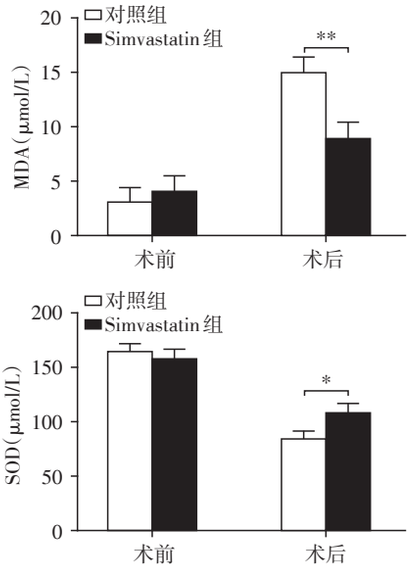
2.5　各组小鼠内质网应激相关蛋白的变化

Western blot显示，与对照组比较，再灌注后Simvastatin组中内质网应激相关蛋白CHOP表达被显著抑制($P<0.05$，图4)。

3　讨　论

肝脏IRI是肝部分切除术和肝移植手术过程中不可避免的病理生理过程，影响患者的预后[12-13]。然而，目前改善肝脏IRI的方法有限。因此，本研究的目的是观察辛伐他汀预处理能否缓解肝部分切除术后的肝脏IRI，并探讨其可能机制。本研究发现辛伐他汀预处理能缓解术后肝脏IRI，表现为血清ALT、AST及TBIL水平降低，组织炎症因子IL-1、IL-6、INOS及TNF-α产生抑制，血清MDA减少和SOD水平上调。

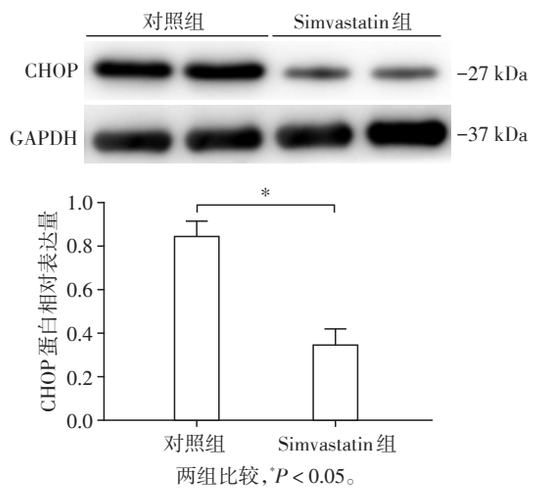
既往研究表明，在肝脏IRI中，细胞缺氧触发氧自由基(reactive oxygen species，ROS)和活性氮自由基(reactive nitrogen species，RNS)等物质释放，募集中性粒细胞等炎症细胞聚集，进而促进蛋白酶分泌增加，加重组织损伤[14-17]。本研究发现辛伐他汀预处理降低了肝脏IRI的氧化应激水平，与对照组相比，Simvastatin组与活性氧积累密切相关的血清MDA水平降低，抗氧化酶SOD增加。



两组比较，$^{*}P<0.05$，$^{**}P<0.01$。

图3　对照组和Simvastatin组患者血清氧化应激水平比较

Figure 3　Comparison of serum oxidative stress levels between control group and Simvastatin group



两组比较，$^{*}P<0.05$。

图4　对照组和Simvastatin组患者肝组织CHOP表达水平比较

Figure 4　Comparison of CHOP expression in liver tissues of patients between control group and Simvastatin group

此外，还探讨了辛伐他汀与内质网应激的关系。内质网作为蛋白质合成的主要细胞器，对刺激高度敏感[18]。内质网应激可导致内质网处理新合成蛋白的能力降低，导致未折叠蛋白在内质网管腔内积累和聚集，参与肝脏IRI等多种病理过程[19-20]。Wada等[21]研究提出，CHOP蛋白作为一种转录因子和内质网应激标志物，在内质网应激诱导的细胞凋亡中起核心作用，且特异性敲除CHOP可增强小鼠对肝脏IRI的抵抗能力[21]。本研究发现，辛伐他汀预处理可明显抑制肝脏缺血再灌注后CHOP蛋白的表达，抑制组织内质网应激水平，缓解肝脏IRI。

综上所述，对于肝细胞癌患者，辛伐他汀预处理能有效缓解肝部分切除术后患者的肝功能损伤，其机制与辛伐他汀预处理抑制组织内氧化应激和内质网应激水平相关。辛伐他汀预处理有希望成为保护肝脏对抗IRI的新方法。

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[收稿日期] 2020-04-19

(上接第1301页)

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