

紫杉醇诱发肺损伤的机制及帕瑞昔布钠的干预作用

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摘要:【目的】探讨紫杉醇诱发大鼠肺损伤的机制及帕瑞昔布钠的干预作用。【方法】实验大鼠随机分为4组:空白对照组(Con组),紫杉醇化疗组(Pac组),帕瑞昔布钠干预组(Pac+Pare组),帕瑞昔布钠组(Pare组)。观察肺泡换气功能、肺泡-毛细血管膜通透性及肺组织病理的改变,检测肺组织炎症因子、环氧合酶2(COX-2)含量及紧密连接蛋白(ZO-1及Claudin-4蛋白)的表达。【结果】与Con组比较,Pac组大鼠肺组织COX-2表达显著升高($P < 0.01$),肺组织炎症改变明显;ZO-1及Claudin-4蛋白表达减少($P < 0.01$);肺泡-毛细血管膜通透性增高($P < 0.01$),肺换气功能减低。Pac+Pare组、Pare组与Con组比较,上述各指标差异无统计学意义。【结论】紫杉醇可损伤大鼠肺泡-毛细血管屏障,其机制可能是紫杉醇引起肺组织COX-2过表达,继而激活炎症级联反应损伤肺泡毛细血管膜上的屏障结构—紧密连接;而COX-2特异性抑制剂帕瑞昔布钠干预可阻断此损伤。

关键词: 肺损伤; 肺泡-毛细血管屏障; 紫杉醇; Cox-2; 紧密连接

中图分类号: R619+.9

文献标志码: A

文章编号: 1672-3554(2013)03-0407-08

Mechanism of Paclitaxel-induced Lung Injury and Preventive Role of Parecoxib

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Abstract: 【Objective】To investigate the mechanism of paclitaxel-induced lung injury and the preventive role of parecoxib in rats. 【Methods】28 Wistar rats were randomly assigned into four groups: Control group, Paclitaxel group, Paclitaxel+Parecoxib group, and Parecoxib group. The changes of pulmonary permeability, gas exchange of alveolar capillary membrane and pathological section were examined. Inflammatory cytokine in lung tissue and the content of COX-2, protein expression of tight junction were detected. 【Results】Compared with the Control group, the Pac group has a significant increase in COX-2 expression, ICAM-1, neutrophils counts and decrease in ZO-1, Claudin-4 protein in lung tissue. Pulmonary permeability in Paclitaxel group was increased and gas exchange dysfunction developed. There were no statistical differences among Paclitaxel+Parecoxib group, Parecoxib group and Control group. 【Conclusion】Paclitaxel chemotherapy injury alveolar capillary barrier in rats. The possible mechanism was that Paclitaxel can cause Cox-2 overexpression, which activated lung inflammatory reaction and damaged the tight junction protein of alveolar capillary membrane. However, the Cox-2 specific inhibitor parecoxib can protect lung from these injuries.

Key words: lung injury; alveolar capillary barrier; paclitaxel; Cox-2; tight junction

[J SUN Yat-sen Univ(Med Sci), 2013, 34(3):407-414]

新辅助化疗是多种恶性实体瘤患者手术前首先采用的治疗措施。有报道,新辅助化疗后,患者肺功能检查时发现一氧化碳弥散功能降低,同时手术后肺部并发症亦显著增多^[1-2],提示新辅助化疗可能诱发了肺损伤。肺泡的弥散依赖于肺泡-毛

细血管屏障功能正常,而上皮细胞间与内皮细胞间的紧密连接(tight junction, TJ)是其重要成分。TJ由Occludin蛋白、ZO(zonula occludens)蛋白、和Claudin蛋白家族组成。当这些蛋白发生变化,可破坏TJ的完整性,引起细胞间连接的开放,导致

收稿日期:2012-11-25

基金项目:广东省自然科学基金(S2011010004049)

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肺泡-毛细血管膜(alveolar capillary membrane ACM)通透性的增加,高蛋白液体及细胞向肺间质及肺泡腔内聚集,引起肺弥散功能障碍^[3-6]。新辅助化疗是否损伤 TJ,继而造成肺损伤,尚无研究报道。紫杉醇是常用的新辅助化疗药。既往研究发现,紫杉醇多程化疗可引起肺泡毛细血管渗漏综合征等肺损伤。此外,还有报道发现紫杉醇化疗可引起肺组织环氧化酶 2(cyclooxygenase-2, COX-2)蛋白表达异常^[7-8]。由于 COX-2 是一种前炎症因子,可激活肺炎性级联反应造成肺损伤,这是否是紫杉醇引起肺损伤的机制?另外,应用 COX-2 特异性抑制剂是否能阻断这种损伤尚需研究。本研究利用单疗程紫杉醇化疗大鼠模型,通过检测肺泡-毛细血管屏障通透性,及肺组织炎症因子、COX-2 及紧密连接蛋白(ZO-1、Claudin-4)的表达等,阐明紫杉醇新辅助化疗诱发肺损伤的特点及机制,同时探讨 COX-2 特异性抑制剂帕瑞昔布钠的干预作用,为防治新辅助化疗患者手术后肺部并发症提供实验依据。

1 材料与方 法

1.1 材 料

健康雌性 Wistar 大鼠 28 只,约 9 周龄,200 ~ 220 g,中山大学南校区实验动物中心提供;紫杉醇注射液(Taxol®, Bristol Myers Squibb, SRL);注射用帕瑞昔布钠(Dynastat®, Pfizer Limited, Sandwich, UK);I-STAT 血气分析仪、EG7+血气分析仪测试卡(美国雅培公司);伊文氏蓝、甲酰胺(美国 Amresco 公司);Claudin-4 单克隆鼠抗(329400)、ZO-1 多克隆兔抗(617300)购于美国 invitrogen 公司,ICAM 单克隆鼠抗(NB500-318)购于 Novus Biologicals, Cox-2 多克隆羊抗(3362-100)购于 Biovision 公司。HRP 标记二抗(美国 santa cruz biotechnology),Bio2radmicrop late 550 酶标仪(美国),UnicamHeliox 紫外可见分光光度计(英国)。

1.2 动物处理

大鼠饲养于中山大学北校区实验动物中心屏障系统。随机将大鼠分为空白对照组(Con 组),紫杉醇化疗组(Pac 组),帕瑞昔布钠干预组(Pac + Pare 组),帕瑞昔布钠组(Pare 组),每组 7 只。按参考文献的方法,Pac 组大鼠连续 6 d 腹腔注射 0.5 mg/mL 紫杉醇 2 mg/kg;Con 组大鼠连续 6 d 腹腔

注射生理盐水 1 mL;Pac + Pare 组大鼠除连续 6 d 腹腔注射紫杉醇 2 mg/kg 外,于第 4 天起,早晚各一次腹腔注射帕瑞昔布钠 2 mg/kg 至第 6 天;Pare 组大鼠前 4 d 连续腹腔注射生理盐水 4 mL/kg。于第 4 天起,早晚各 1 次腹腔注射帕瑞昔布钠 2 mg/kg 至第 6 天。大鼠正常饮食、饮水。于给药后第八天麻醉下放血处死大鼠。

1.3 标本采集

1.3.1 动脉血气分析 大鼠 10%水合氯醛 3 mL/kg 腹腔注射,固定后右颈动脉采血 2 mL,取少许进行血气分析。余血离心(3000 r/min, $r = 15$ cm, 15 min),取上清 BCA (Bicinchoninic acid)法测蛋白浓度。

1.3.2 肺组织采集及病理评分 放血处死大鼠,打开胸腔,切出右肺,右中肺叶经 40 g/L 多聚甲醛溶液固定,石蜡切片 HE 染色后光镜观察,用四级评分法对肺组织炎性改变、肺泡腔出血、肺泡壁水肿增厚进行评价(0 分为无病理改变;1 分有轻度改变,面积 30% 以下;2 分为中度改变,30% ~ 60%;3 分为重度改变,60% 以上)。余下右肺冻存于液氮罐留待组织检测。

1.3.3 肺泡灌洗、细胞计数及肺通透指数的测定

结扎右主支气管,进行左侧肺泡灌洗,切开气管,经气管插入 12 号针头,缝线结扎固定以防肺泡灌洗液(Broncho-alveolar lavage fluid, BALF)溢出,无菌 4 ℃ 生理盐水 5 mL 分 3 次经导管缓慢注入,反复抽吸,总回收量应在 3.8 mL 以上,最后将动物置于头低足高位。BALF 置于无菌离心管内离心(1 500 r/min, $r = 15$ cm, 10 min, 4 ℃),取上清 BCA 法测定 BALF 中蛋白浓度,并计算肺通透指数(BALF 中蛋白浓度/血清蛋白浓度, lung permeability index, LPI)。沉淀细胞用生理盐水洗涤离心 2 次,加入生理盐水 1 mL,吹打混匀成细胞悬液,置于改良牛氏计数板进行细胞计数。沉渣细胞涂片行 HE 染色,至少计数 200 个细胞做细胞分类计数。

1.3.4 伊文氏蓝测定肺通透性 每组另 3 只大鼠取动脉血后经颈静脉注射 6 mg/mL 伊文氏蓝溶液 30 mg/kg, 30 min 后开胸剪开右房,100 mL 生理盐水经右室以 20 cm H₂O 压力冲洗肺循环,取出双肺,剪去气管,清洗擦干肺组织,称重后匀浆,以每 100 mg 肺组织 1 mL 的甲酰胺浸泡肺匀浆,60 ℃ 下孵浴 18 h, 12 000 r/min($r = 6$ cm)离心 20 min,

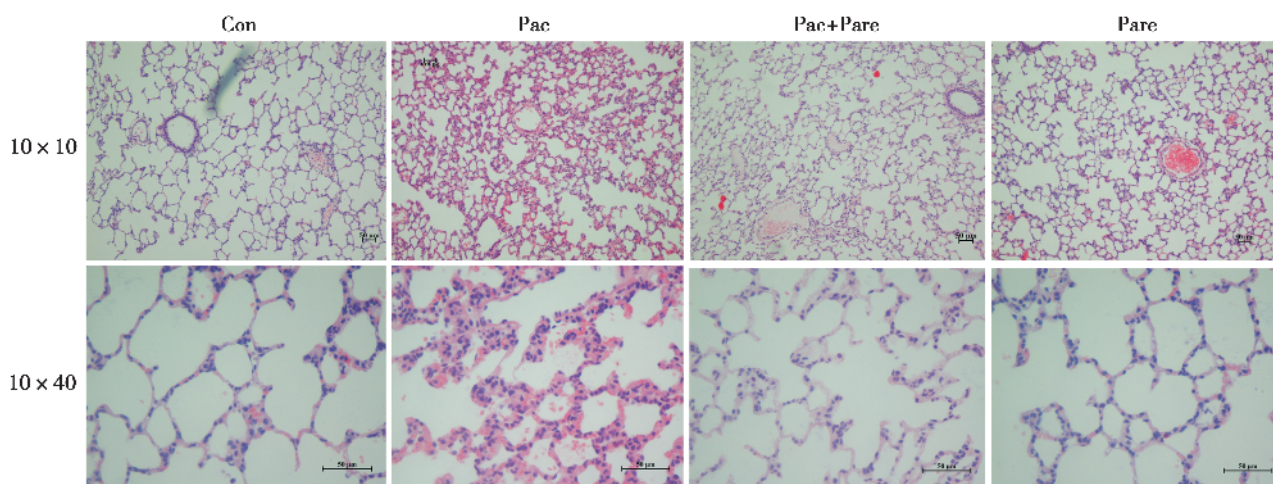


图1 四组肺组织学观察(HE染色)

Fig.1 Comparison of lung pathological section(HE staining)

Con group was normal lung tissue. The structure of pulmonary alveoli was clear and the alveolar wall was thin. Pac group has a severe inflammation, the alveolar structure was in disorder, there were edema and broadening in lung interstitium while hemorrhage in both alveolar cavity and interstitium. Pac+Pare group was significantly slighter compared with Pac group and it seemed only a little edema. Pare group was more similar to Con group. ($n = 4$).

取上清液于 620 nm 波长测得吸光度,根据甲酰胺标准曲线计算肺组织甲酰胺含量。

1.4 检测肺组织中 COX-2、ICAM-1、ZO-1、Claudin-4 蛋白表达 取冻存肺组织,用冰生理盐水漂洗,除去血液,滤纸拭干。称取 100mg 肺组织,将组织块放入研钵内尽快用眼科剪剪碎,加入液氮,磨碎至糊状加入 1 mL 蛋白裂解液,冰上放置 20 min 后离心,12 000 r/min 4 ℃ 离心 30 min,取上清再次离心 15 min。按试剂盒说明书,采用蛋白免疫印迹检测肺组织中 COX-2、胞间粘附因子-1 (intercellular adhesion molecule-1, ICAM-1)、ZO-1、Claudin-4 蛋白表达。

1.5 统计学方法

运用 SPSS 13.0 软件包进行统计分析。数据以 $\bar{x} \pm s$ 表示。多组样本间均数比较采用方差分析,两组样本间均数比较采用成组 t 检验。 $P < 0.05$ 表示差异有统计学意义。

2 结果

2.1 各组肺组织学观察

Con 组肺组织结构清晰完整,无炎性细胞浸润,无出血,肺泡壁无水肿增厚;Pac 组肺泡结构紊乱,有明显炎细胞渗出,多处肺泡腔内可见散在红细胞,肺间质水肿增厚;Pac+Pare 组的病理损害

明显较 Pac 组减轻,肺泡结构清楚,水肿及增厚不明显,炎症显著减弱;Pare 组病理结果与 Con 组相似,见图 1。病理评分 Pac 组与其余三组皆有显著差异, $P < 0.01$,而 Con 组、Pac + Pare 组与 Pare 组之间比较则无统计差异(表 1)。

2.2 各组肺组织伊文氏蓝含量比较

肉眼见 Pac 组肺组织明显蓝于其余 3 组,见图 2。检测结果 Con 组依文氏蓝含量为 (108 ± 12.1) ng/每克肺组织,Pac 组、Pac + Pare 组、Pare

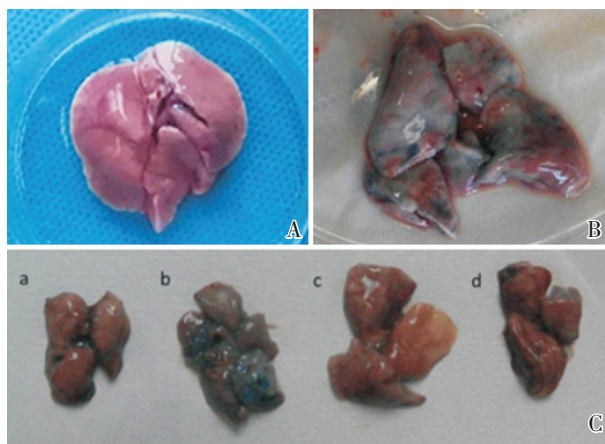


图2 正常肺组织和伊文氏蓝染色肺组织

Fig.2 Normal lung and lung stained with Evans Blue

A: Normal lung; B: Pac group lung after Evans Blue iv; C. a, b, c, d were lungs in turns from Con, Pac, Pac+Pare and Pare group rats 30min after Evans Blue iv.

表 1 各组肺组织病理评分

Table 1 Pathological score of lung in each group

	Con	Pac	Pac+Pare	Pare
Score	0.5 ± 0.15	2.6 ± 0.29 ¹⁾	0.7 ± 0.25 ²⁾	0.6 ± 0.14 ²⁾

1) compared with the other groups, $P < 0.01$; 2) compared with Con group, there was no significant difference. Pare group is of no difference with Pac+Pare group. Data were presented as $\bar{x} \pm s$, ($n = 4$)

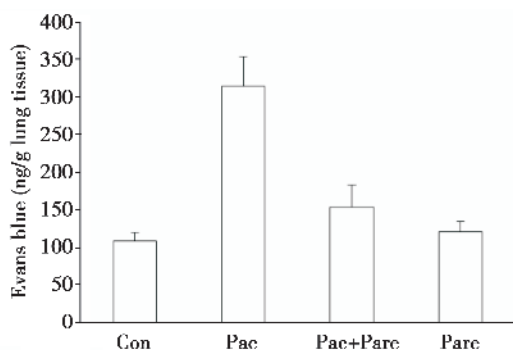


图 3 肺组织中渗入伊文氏蓝的含量

Fig.3 Content of Evans Blue in every gram lung tissue

Evans Blue in Con group was (108 ± 12.1)ng/g, and compared with other groups, $P < 0.01$. There were no significant differences among Con group, Pac+Pare group (154 ± 28.6)ng/g and Pare group (120 ± 13.5)ng/g. Data were presented as $\bar{x} \pm s$, ($n = 3$).

组分别为(314.3 ± 38.7)ng/g 肺组织、(154 ± 28.6)ng/g 肺组织、(120.7 ± 13.5)ng/g 肺组织。Pac 组显著高于其余三组($P < 0.01$),其余 3 组两两比较差异无统计学意义($P > 0.05$,图 2、3)。

2.3 各组动脉血气参数比较

与其它组比较,Pac 组动脉血 PCO_2 显著升高, PO_2 及 SaO_2 显著降低,差异有统计学意义 ($P < 0.01$)。Pac + Pare 组、Pare 组与 Con 组比较上述各指标差异无统计学意义($P > 0.01$,图 4、表 2)。

2.4 各组肺通透指数比较

四组 BALF 的回收率在 87%以上, 各组之间差异无统计学意义。Pac 组肺通透指数 (2.2 ± 0.56)%明显高于其余 3 组($P < 0.01$)。帕瑞昔布钠干预组 (0.97 ± 0.21)%及帕瑞昔布钠 (0.77 ± 0.24)%与对照组(0.72 ± 0.22)%比较,差异无统计学意义($P > 0.05$,图 5)。

2.5 各组 BALF 中细胞计数比较

BALF 中总白细胞计数及中性粒细胞分类计数结果显示 Pac 组细胞数较其他三组明显增高($P < 0.01$),且以中性粒细胞增高为主。Pac + Pare 组,

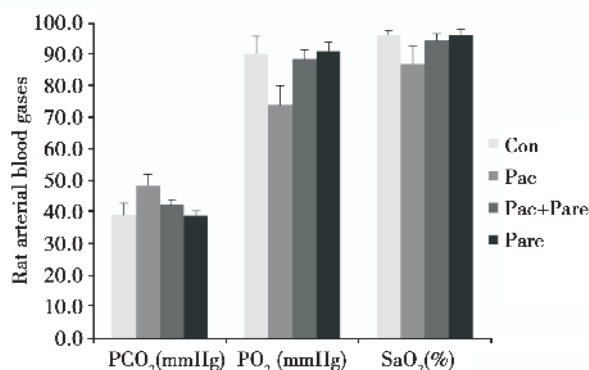


图 4 不同处理对大鼠动脉血气值的影响

Fig.4 The effect of different treatment to rat arterial blood gases

The PCO_2 of Pac group was significantly higher compared with other groups while PO_2 and SaO_2 decreased, $P < 0.01$; There were no statistical differences among Con group, Pac+Pare group and Pare group in the three indicators. Data were presented as $\bar{x} \pm s$, ($n = 7$).

表 2 血气分析结果

Table 2 The data of arterial blood gas analysis

	PCO_2 (mmHg)	PO_2 (mmHg)	SaO_2 (%)
Con	39 ± 3.8	90.2 ± 5.3	96 ± 1.5
Pac	48.3 ± 3.6 ¹⁾	73.8 ± 6.0 ¹⁾	86.8 ± 5.9 ¹⁾
Pac+Pare	42.3 ± 1.5 ²⁾	88.3 ± 3.1 ²⁾	94.3 ± 2.1 ²⁾
Pare	38.9 ± 1.5 ²⁾	91 ± 2.6 ²⁾	96 ± 1.7 ²⁾

1) compared with others, $P < 0.01$; 2) compared with Con group, there was no significant difference. Pare group is of no difference with Pac+Pare group. Data were presented as $\bar{x} \pm s$, ($n = 7$).

Pare 组及 Con 组之间比较差异无统计意义 ($P > 0.05$,图 6)。

2.6 Western Blot 检测 ICAM-1、COX-2、ZO-1 及 Claudin-4 蛋白

Con 组 COX-2、ICAM-1 表达微弱, 而 ZO-1 及 Claudin-4 表达正常;Pac 组 COX-2、ICAM-1 明显增高,同时 ZO-1、Claudin-4 表达减低, $P < 0.01$ 。Pac+Pare 组及 Pare 组 COX-2、ICAM-1 正常, 且 ZO-1、Claudin-4 表达恢复, 与 Pac 组有差异, $P < 0.01$ 。Pac + Pare 组、Pare 组及 Con 组之间比较差异无统计意义($P > 0.05$,图 7)。

3 讨 论

本实验以每日 2 mg/kg 紫杉醇单疗程给予大

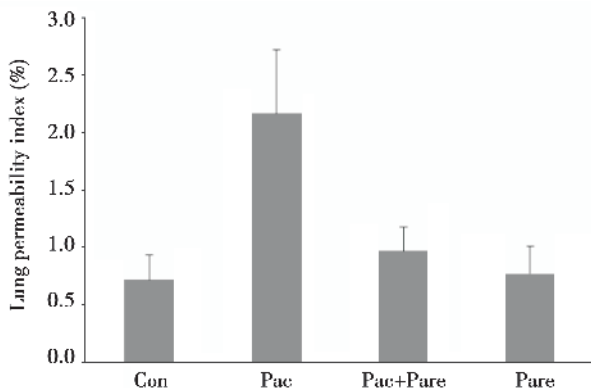


图5 四组大鼠肺通透性指数(BALF蛋白/血清蛋白)的变化

Fig.5 The change of lung permeability index (LPI, BALF protein/serum protein) in different groups

The Pac group (2.2 ± 0.56)% was statistically more permeable than Con group (0.72 ± 0.22)%, Pac+Pare group (0.97 ± 0.21)%, Pare group (0.77 ± 0.24)%, $P < 0.01$. There were no statistical differences among Con group, Pac+Pare group and Pare group. Data were presented as $\bar{x} \pm s$, ($n = 4$).

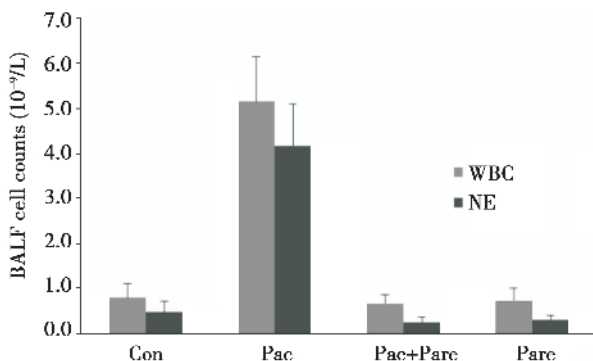


图6 肺泡灌洗液白细胞及中性粒细胞计数

Fig.6 The count of WBC and NE in BALF

The total WBC was sharply increasing in Pac group, which was dominated by NE. In other three groups, there were less cells and NE occupied less percentage, $P < 0.01$. Data were presented as $\bar{x} \pm s$, ($n = 4$). WBC; white blood cell; NE; neutrophils.

鼠腹腔注射模拟新辅助化疗病人肺损伤病理生理改变。实验结果显示:①Pac组病理切片显示肺泡间隔水肿渗出增厚、炎细胞浸润、肺泡腔小片状红细胞,提示炎症反应参与损伤过程,证实了肺泡毛细血管膜(ACM)对水和细胞的通透性增高;②Pac组肺组织伊文氏蓝含量及肺通透指数增高,这两项结果提示 ACM 的物理屏障、电化学屏障功能都受到损伤,对大分子及带电荷分子的通透性增高;③血气分析结果 Pac 组 PCO_2 升高, PO_2 及 SO_2 降

低,与 Con 组有显著的统计学差异,说明紫杉醇损伤 ACM,进而影响了气体交换。这三组指标都是反映 ACM 通透性的,其变化趋势也是一致的,从三个角度共同展示了 Pac 组应用紫杉醇后 ACM 屏障功能受损的表现:通透性显著升高,不能调控物质的跨膜转运,毛细血管中的水、蛋白、细胞及伊文氏蓝染料等自由向肺泡间质及肺泡腔渗入,导致 ACM 增厚, CO_2 和 O_2 弥散减慢。

肺换气功能(CO_2 和 O_2 正常弥散)依赖于肺泡腔和肺毛细血管腔相互隔离及 ACM 维持在一定的厚度之内,这取决于肺泡毛细血管膜屏障功能的完善。紧密连接(tight junction, TJ)是相邻上皮/内皮细胞游离端侧壁间的胞膜蛋白形成的复合体,在细胞内和细胞骨架相互交联,调节经细胞旁途径的水、离子及大分子的转运,是保障肺泡毛细血管屏障功能的重要结构,其主要组成为 Occludin 蛋白、ZO 蛋白、和 Claudin 蛋白家族。ZO-1、Claudin-4 是 TJ 较为重要的组分,其表达量降低在多种组织上皮/内皮损伤及通透性增高的研究中都发挥关键作用^[3,9-10]。本实验 Pac 组应用紫杉醇后 TJ 蛋白 ZO-1、Claudin-4 表达降低,与该组 ACM 屏障受损、通透性增高及 CO_2 和 O_2 弥散障碍的表现是相一致的,首次证实了紫杉醇对 ACM 的损伤与其影响了 TJ 有关。这与 Mitchell LA 等提到的 TJ 在维持肺泡毛细血管屏障中发挥重要作用^[3]及 Wray C 等论证的 Claudin-4 在急性肺损伤、机械通气肺损伤中对 ACM 通透性改变的保护作用^[4]是一致的。我们认为紫杉醇抑制了 ZO-1、Claudin-4 的表达,影响 TJ 的完整性,从而造成肺泡毛细血管屏障功能障碍,ACM 通透性增高,毛细血管中的高蛋白液体及细胞渗入肺泡间隙甚至肺泡腔内,造成肺水肿,最终 ACM 增厚,致气体弥散障碍^[5-6]。

本实验中紫杉醇减低 TJ 组分表达与其增强组织 COX-2 含量具有一致性,提示高表达的 COX-2 可能参与紫杉醇损伤 TJ 的过程并发挥了重要作用。1)COX-2 是诱导型环氧合酶,多种细胞包括中性粒细胞可产生,并能抑制中性粒细胞凋亡,与中性粒细胞可相互促进引起炎症反应的放大和增强,产生细胞毒效应,在肺损伤中有重要意义^[11-14]。ICAM-1 可由炎症介质诱导在多种结构细胞上表达,参与介导中性粒细胞与结构细胞黏附,对中性粒细胞行使功能至关重要^[15-16]。在紫杉

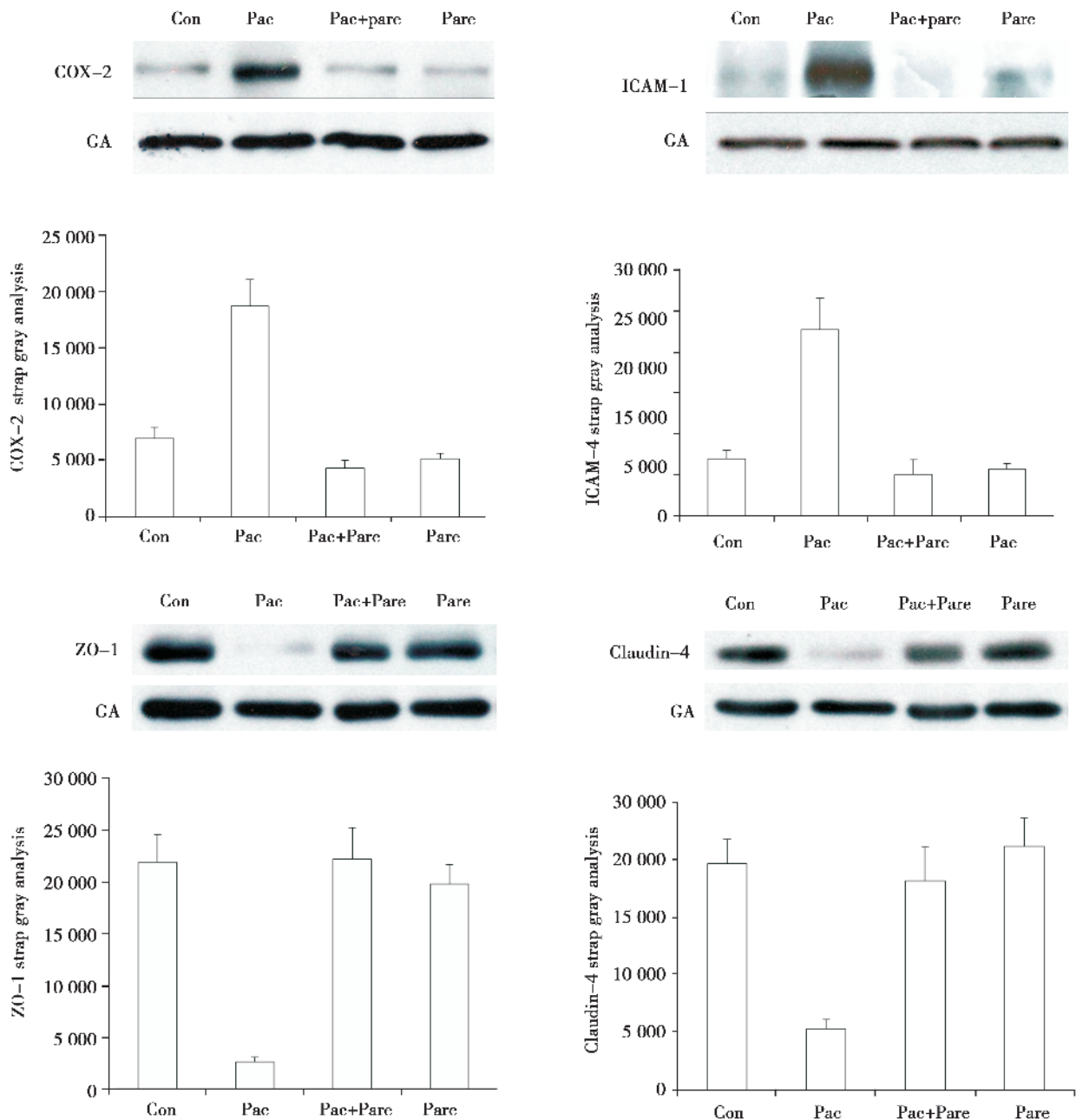


图 7 不同处理后肺组织中 COX-2、ICAM-1、ZO-1、Claudin-4 蛋白表达的变化

Fig.7 COX-2, ICAM-1, ZO-1, and Claudin-4 protein expression in rat lung tissue after different treatment in each group

Immunoblotting was showing increase in COX-2, ICAM-1 and reduction in ZO-1, Claudin-4 after given one course of paclitaxel compared with normal lung, $P < 0.01$. These changes were reversed by using parecoxib while parecoxib only resembled Con group. The bar-graph under the protein strap represented corresponding band intensities, data were presented as $\bar{x} \pm s$ and corrected by GA. ($n = 4$).

醇耐药性研究中曾证实紫杉醇可引起上皮细胞和内皮细胞 COX-2 mRNA 表达及 COX-2 水平的增高^[7-8],这可能是紫杉醇刺激了 COX-2 的转录作用及增强了 mRNA 的稳定性^[17]。本实验 Pac 组大鼠肺病理切片呈炎性反应,中性粒细胞浸润;肺泡

灌洗液中性粒细胞数量和比例明显升高;组织匀浆中 COX-2、ICAM-1 增高,这些指标都是肺部炎症反应的表现,印证了紫杉醇可调强肺组织 COX-2 表达,并引发/放大炎症反应。②在多种组织的 TJ 功能研究中,常发现 TJ 功能减弱、蛋白表达减

低与 COX-2 高表达呈线性关系^[18-20],但目前的研究尚未证实两者是否存在因果关系。本实验应用特异性 COX-2 抑制剂帕瑞昔布钠后肺组织 COX-2、ICAM-1 含量下降、病理切片和灌洗液显示炎症反应减轻; TJ 蛋白 ZO-1 和 Claudin-4 表达恢复; 反映肺组织高通透状态的各项指标均降低并且肺换气功能指标 PCO₂、PO₂ 及 SaO₂ 接近正常; 证实了紫杉醇所致肺损伤是通过其引发 COX-2 高表达, 引发/放大炎症反应, 影响了 TJ 蛋白而实现的, 应用 COX-2 抑制剂帕瑞昔布可逆转紫杉醇对 ACM 的损伤。

新辅助化疗常可增加手术风险, 引起术后肺部并发症, 有研究发现术前肺功能检查 D_{lco}% 和 D_{lco}/V_a% 的减低可提示手术风险, 预测并发症, 机制尚未清楚, 但可能与肺炎及肺渗透性增加有关^[1-2], 手术推迟至 D_{lco}% 和 D_{lco}/V_a% 恢复则手术风险可有效降低^[1]。常用药物紫杉醇等可影响肺功能, 尤其是 D_{lco}^[21], D_{lco} 即一氧化碳弥散量, 是反映 ACM 换气功能的金标准, 各种原因引起的 ACM 增厚和纤维化都可造成 D_{lco} 减低。在本实验中, 我们证实紫杉醇破坏了肺泡毛细血管屏障而致 ACM 渗出增厚, 从而影响气体弥散, 具体机制是 COX-2 介导的中性粒细胞参与的炎症反应, 其特异性抑制剂可拮抗紫杉醇引起的 TJ 减少及 ACM 损伤。

紫杉醇新辅助化疗引起的 ACM 弥漫性损伤及术后肺部并发症广泛存在于临床, 与疗程和积累剂量相关, 但尚未有有效的治疗方法。本实验为其提供了一种新的思路, 新辅助化疗联合应用 COX-2 抑制剂以减轻 ACM 损伤, 但仍需临床二期试验提供确凿证据。曾有类似研究证实补骨脂次素(COX-2 及 5-LOX 双重抑制剂)可减轻放疗引起的肺炎^[22]。目前关于帕瑞昔布钠治疗紫杉醇致肺损伤的研究少见报道; 对治疗其他类型肺损伤, 如多器官功能衰竭、烧伤及胰腺炎引发的急性肺损伤^[11-13]帕瑞昔布钠有效, 与高表达的 COX-2 密切相关。

紫杉醇引起的 COX-2 升高与其产生耐药性相关, COX-2 抑制剂可显著减低紫杉醇耐药现象, 增强肿瘤敏感性。亦有临床研究显示 COX-2 有促血管生成作用, 可加速肿瘤的生长转移^[23,24], 甚至作为多种肿瘤预后的标记物, 临床试验证实紫杉醇联合抗 COX-2 治疗常可取得好的抗肿瘤

效果^[7, 23,25-26]。而我们的研究证实了 COX-2 抑制剂除外抗肿瘤作用, 还可减轻紫杉醇新辅助化疗引起的 ACM 损伤, 为推广术前化疗联合 COX-2 抑制剂的应用方案提供了更多依据。

参考文献:

- [1] Cerfolio RJ, Talati A, Bryant AS. Changes in pulmonary function tests after neoadjuvant therapy predict postoperative complications [J]. *Ann Thorac Surg*, 2009, 88(3): 930-935.
- [2] Leo F, Solli P, Spaggiari L, et al. Respiratory function changes after chemotherapy: an additional risk for postoperative respiratory complications? [J] *Ann Thorac Surg*, 2004, 77(1): 260-265.
- [3] Mitchell LA, Overgaard CE, Ward C, et al. Differential effects of claudin-3 and claudin-4 on alveolar epithelial barrier function [J]. *Am J Physiol Lung Cell Mol Physiol*, 2011, 301(1): L40-L49.
- [4] Wray C, Mao Y, Pan J, et al. Claudin-4 augments alveolar epithelial barrier function and is induced in acute lung injury [J]. *Am J Physiol Lung Cell Mol Physiol*, 2009, 297(2): L219-L227.
- [5] Coyne CB, Gambling TM, Boucher RC, et al. Role of claudin interactions in airway tight junctional permeability [J]. *Am J Physiol Lung Cell Mol Physiol*, 2003, 285(5): L1166-L1178.
- [6] Lê BV, Khorsi-Cauet H, Bach V, et al. Mast cells mediate *Pseudomonas aeruginosa* lipopolysaccharide - induced lung inflammation in rat [J]. *Eur J Clin Microbiol Infect Dis*, 2012, 31(8): 1983-1990.
- [7] Altorki NK, Port JL, Zhang F, et al. Chemotherapy induces the expression of cyclooxygenase -2 in non - small cell lung cancer [J]. *Clin Cancer Res*, 2005, 11(11): 4191-4197.
- [8] Huang WZ, Fu JH, Wang DK, et al. Overexpression of cyclooxygenase -2 is associated with chemoradiotherapy resistance and prognosis in esophageal squamous cell carcinoma patients [J]. *Dis Esophagus*, 2008, 21(8): 679-684.
- [9] Jacob AM, Gaver DP 3rd. Atelectrauma disrupts pulmonary epithelial barrier integrity and alters the distribution of tight junction proteins ZO-1 and claudin 4 [J]. *J Appl Physiol*, 2012, 113(9): 1377-1387.
- [10] Li R, Ning Z, Cui J. Diesel exhaust particles modulate vascular endothelial cell permeability: implication of ZO-1 expression [J]. *Toxicol Lett*, 2010, 197(3): 163-168.

- [11] Ang SF, Sio SW, Mochhala SM, et al. Hydrogen sulfide upregulates cyclooxygenase-2 and prostaglandin E metabolite in sepsis-evoked acute lung injury via transient receptor potential vanilloid type 1 channel activation[J]. *J Immunol*, 2011, 187(9): 4778-4787.
- [12] Sio SW, Ang SF, Lu J, et al. Substance P upregulates cyclooxygenase-2 and prostaglandin E metabolite by activating ERK1/2 and NF- κ B in a mouse model of burn-induced remote acute lung injury[J]. *J Immunol*, 2010, 185(10): 6265-6276.
- [13] O'Brien G, Shields CJ, Winter DC, et al. Cyclooxygenase-2 plays a central role in the genesis of pancreatitis and associated lung injury[J]. *Hepatobiliary Pancreat Dis Int*, 2005, 4(1): 126-129.
- [14] Jinzhou Z, Tao H, Wensheng C, et al. Cyclooxygenase-2 suppresses polymorphonuclear neutrophil apoptosis after acute lung injury[J]. *J Trauma*, 2008, 64(4): 1055-1060.
- [15] Kira S, Daa T, Kashima K, et al. Mild hypothermia reduces expression of intercellular adhesion molecule-1 (ICAM-1) and the accumulation of neutrophils after acid-induced lung injury in the rat [J]. *Acta Anaesthesiol Scand*, 2005, 49(3): 351-359.
- [16] Lundberg AH, Fukatsu K, Gaber L. Blocking pulmonary ICAM-1 expression ameliorates lung injury in established diet-induced pancreatitis [J]. *Ann Surg*, 2001, 233(2): 213-220.
- [17] Subbaramaiah K, Marmao TP, Dixon DA, et al. Regulation of cyclooxygenase-2 mRNA stability by taxanes: evidence for involvement of p38, MAPKAPK-2 and HuR[J]. *J Biol Chem*, 2003, 278(39): 37637-37647.
- [18] In-ah L, Dong-hyun K. *Klebsiella pneumoniae* increases the risk of inflammation and colitis in a murine model of intestinal bowel disease [J]. *Scandinavian Journal of Gastroenterology*, 2011, 46(6): 684-693.
- [19] Hung CF, Fang CL, Al-Suwayeh SA, et al. Evaluation of drug and sunscreen permeation via skin irradiated with UVA and UVB: Comparisons of normal skin and chronologically aged skin[J]. *J Dermatol Sci*, 2012, 68(3): 135-148.
- [20] Tai HY, Tam MF, Chou H, et al. Pen ch 13 allergen induces secretion of mediators and degradation of occludin protein of human lung epithelial cells [J]. *Allergy*, 2006, 61(3): 382-388.
- [21] Dimopoulou I, Galani H, Dafni U, et al. A prospective study of pulmonary function in patients treated with paclitaxel and carboplatin[J]. *Cancer*, 2002, 94(2): 452-458.
- [22] Yang HJ, Youn H, Seong KM. A dual inhibitor of COX-2 and 5-LOX, regulates ionizing radiation (IR)-induced pulmonary inflammation [J]. *Biochem Pharmacol*, 2011, 82(5): 524-534.
- [23] Bamias A, Kyriakou F, Chorti M. Microvessel density (MVD) and cyclooxygenase-2 (COX-2)/beta-catenin interaction are associated with relapse in patients with transitional carcinoma receiving adjuvant chemotherapy with paclitaxel/carboplatin; a hellenic cooperative oncology group (HECOG) study [J]. *Anticancer Res*, 2008, 28(4C): 2479-2486.
- [24] Wang W, Bergh A, Damber JE. Cyclooxygenase-2 expression correlates with local chronic inflammation and tumor neovascularization in human prostate cancer [J]. *Clin Cancer Res*, 2005, 11(9): 3250-3256.
- [25] Dandekar DS, Lopez M, Carey RI, et al. Cyclooxygenase-2 inhibitor celecoxib augments chemotherapeutic drug-induced apoptosis by enhancing activation of caspase-3 and -9 in prostate cancer cells [J]. *Int J Cancer*, 2005, 11(3): 484-492.
- [26] Altorki NK, Christos P, Port JL, et al. Preoperative taxane-based chemotherapy and celecoxib for carcinoma of the esophagus and gastroesophageal junction: results of a phase 2 trial[J]. *J Thorac Oncol*, 2011, 6(6): 1121-1127.

(编辑 张恩健)