

乌司他丁干预对水下爆炸致兔急性肺损伤 TNF- α 表达的影响*

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摘要:目的 探讨乌司他丁干预对水下爆炸致兔急性肺损伤时肿瘤坏死因子(tumor necrosis factor- α , TNF- α)的影响。方法 健康新西兰兔随机分为爆炸损伤组与乌司他丁治疗组,以水下爆炸致兔急性肺损伤,用免疫酶联法检测爆炸后4, 12和24 h血清中TNF- α 浓度。以SPSS17.0统计软件进行分析, $P < 0.05$ 为差异具有统计学意义。结果 爆炸后4 h乌司他丁组TNF- α 浓度为 386.90 ± 109.22 ng/L,爆炸损伤组TNF- α 浓度为 538.20 ± 201.43 ng/L,二者相比无变化($t = 2.088$, $P = 0.051$);爆炸后12 h乌司他丁组TNF- α 浓度为 400.60 ± 78.98 ng/L,爆炸损伤组TNF- α 浓度为 573.80 ± 178.24 ng/L,二者相比差异有统计学意义($t = 2.809$, $P = 0.012$);24 h乌司他丁组TNF- α 浓度为 356.10 ± 130.99 ng/L,爆炸损伤组为 552.30 ± 169.64 ng/L,二者相比差异也有统计学意义($t = 2.895$, $P = 0.010$)。结论 乌司他丁干预下实验兔在爆炸12 h后血清TNF- α 降低。

关键词:水下爆炸;急性肺损伤;乌司他丁;肿瘤坏死因子- α

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Effect of Ulinastatin on the Expression of TNF- α in the Rabbits with Acute Lung Injury Induced by Underwater Explosion

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Abstract: Objective The expression of TNF- α was detected in sera of rabbits treated by ulinastatin with acute lung injury induced by underwater explosion. **Methods** Rabbits were randomly divided into two groups such as the injured group and ulinastatin therapy group. Established underwater explosion device was used to cause acute lung injury in rabbits. TNF- α in sera of the rabbits were measured by ELISA at 4, 12 and 24 hours after the explosion. The SPSS17.0 software was used to analyze the data and $P < 0.05$ was considered to be significant. **Results** There was no significant difference between the concentrations of TNF- α in the sera of rabbits in the injury group (538.20 ± 201.43 ng/L) and that of in the ulinastatin group (386.90 ± 109.22 ng/L, $t = 2.088$, $P = 0.051$) at 4 hours after burst. However, there was evidently decreased in the level of TNF- α in the ulinastatin group (400.60 ± 78.98 ng/L) compared with the injury group (573.80 ± 178.24 ng/L, $t = 2.809$, $P = 0.012$) at 12 hours after burst. Moreover and TNF- α in the ulinastatin group (356.10 ± 130.99 ng/L) was also decreased compared to the injury group (552.30 ± 169.64 ng/L, $t = 2.895$, $P = 0.010$) at 24 hours after burst. **Conclusion** The TNF- α expression in sera of the rabbits in ulinastatin group were dramatically decreased than that of in injury group at 12 hours after burst, which may be benefit to rabbits with acute lung injury induced by underwater explosion.

Keywords: underwater explosion; acute lung injury; ulinastatin; tumor necrosis factor- α

一般认为,爆炸致胸部、腹部或脊髓等损伤较多^[1],而水下爆炸伤是岛礁作战和渡海登陆作战中常见的一种损伤,水下冲击伤以肺部损伤最为严重,是伤者死亡的主要原因之一^[2]。但目前对水下爆炸冲击波致心肺损伤及救治的研究甚少。有文献提示空气爆炸伤引起的急性肺损伤(acute lung injury, ALI)是由多种炎性递质及效应细胞共同

参与,在参与炎症反应与肺损伤中的众多炎症递质中,肿瘤坏死因子- α (tumor necrosis factor- α , TNF- α)与ALI的发病和严重程度密切相关,对该细胞因子的研究将有助于ALI患者的治疗^[3,4]。乌司他丁是一种胰蛋白酶抑制剂,可抑制炎症因子的释放,阻止急性肺损伤的发生发展,有效缓解急性炎症引起的肺损害^[5,6]。因此,ALI时给予乌司

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他丁治疗可能有助于患者的恢复。基于此,我们通过监测水下爆炸致 ALI 时兔血清 TNF- α 变化探讨乌司他丁治疗对 TNF- α 表达的影响,从而为肺损伤的致病机制和临床救治提供有益参考。

1 材料与方法

1.1 研究对象 健康成年新西兰兔 20 只(安徽长临河医药科技有限公司,许可证号 syxk(皖)2007-002),体重 1.8 ± 0.2 kg。动物实验前适应性喂养 3 天,自由饮食饮水,观察一般情况及生命体征无异常即可用于实验。

1.2 试剂和仪器 DNM-9602 酶标分析仪(北京普朗);乌司他丁(广东天普生化医药股份有限公司,批号 031505264);兔 TNF- α ELISA 试剂盒(上海江莱生物科技有限公司,批号 201602)。

1.3 实验方法 将动物随机分为爆炸损伤组(10 只)和乌司他丁治疗组(用量 2.5 万单位/kg 体重,10 只)。实验前动物禁食 6 h,按照文献和前期试验结果麻醉和程序操作^[7]。乌司他丁治疗组实验兔在爆炸后即刻通过静脉置管给予药物,爆炸损伤组实验兔给予同等剂量生理盐水。在爆炸后 4, 12, 24 h 取血后静置 2 h 后分离血清,经 4 000 r/min 离心 10 min,吸取血清至 EP 试管内, -40℃ 冰箱冻存储备用。采用 ELISA 试剂盒检测 TNF- α 含量,严格按照说明书进行。具体步骤:先加稀释液 40 μ l,然后再加待测样品 10 μ l,空白孔以 PBS 溶液代替,37℃ 温育 30 min;洗涤五次后每孔加入酶标液 50 μ l,空白孔除外,37℃ 温育 30 min;再次洗涤后加入显色液,37℃ 避光显色 15 min,最后在 10 min 内测定每孔在 450 nm 处 A 值,依据标准曲线计算 TNF- α 含量。

1.4 统计学分析 数据采用 SPSS17.0 统计软件处理, $P < 0.05$ 为差异有统计学意义。

2 结果 见表 1。为探讨炎性因子 TNF- α 在爆炸性肺损伤中的可能作用,我们采用 ELISA 方法对爆炸损伤组和乌司他丁治疗组兔血清 TNF- α 浓度进行了检测,结果显示乌司他丁治疗组与爆炸损伤组相比血清 TNF- α 在爆炸后 4 h 未见改变,但 12 h 和 24 h 乌司他丁治疗组血清 TNF- α 均降低,提示乌司他丁干预治疗后 TNF- α 的降低可能有利于机体发挥保护作用,减少炎症损伤。

表 1 各组在不同时间点血 TNF- α 浓度的比较($\bar{x} \pm s$, ng/L)

时间(h)	治疗组(n=10)	损伤组(n=10)	t	P
4	386.90 \pm 109.22	538.20 \pm 201.43	2.088	0.051
12	400.60 \pm 78.98	573.80 \pm 178.24	2.809	0.012
24	356.10 \pm 130.99	552.30 \pm 169.64	2.895	0.010

3 讨论 水下爆炸伤时,肺是柔软与含气组织,最易受到冲击,是主要的受损靶器官^[8]。在肺损伤过程中,多种炎症因子参与其中,其中 TNF- α 可激活信号分子 NF- κ B 并触发过度的炎症反应^[9],在治疗上,有效控制炎症因子的过度分泌和肺部炎症反应是治疗 ALI 的关键^[10]。乌司他丁能够抑制氧自由基产生,抑制白细胞过度激活、抑制血管通透性增加,是常用的急性炎症反应治疗药物^[11]。我们通过在爆炸即刻给予乌司他丁干预,结果显示 12 h 后 TNF- α 浓度显著下降,提示使用乌司他丁可能抑制或减少血清 TNF- α 的释放,有助于创伤性炎症的好转,与该炎症因子作用的报道相似^[12]。乌司他丁还可以通过抑制蛋白水解酶活性,减少组织细胞破坏产生的炎症刺激物对巨噬细胞的进一步激活而降低血清 NF- κ B^[13],而 TNF- α 是 NF- κ B 的上游启动子,降低 NF- κ B 的转录翻译可以达到降低炎症反应的作用^[9]。乌司他丁在水下爆炸致兔急性肺损伤早期使用明显降低 TNF- α 的表达,可能在预防肺损伤不良预后发生中扮演着重要作用。

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