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# 乌司他丁治疗水下爆炸致兔急性肺损伤对细胞因子表达的影响

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## 摘　要

**目的：探讨血清肿瘤坏死因子-$\alpha$及中性粒细胞弹性蛋白酶等细胞因子在乌司他丁治疗水下爆炸致兔急性肺损伤时的表达时相变化。方法：将用于实验的动物随机分为对照组、损伤非治疗组、乌司他丁(高、低剂量)治疗组。复制急性肺损伤动物模型，在发生爆炸后立即进行治疗。在治疗4 h，12 h，24 h时取血检测相关细胞因子的浓度。结果：低剂量乌司他丁治疗组在爆炸后12 h (400.6 ± 79.0 ng/L)、24 h (356.4 ± 181.2 ng/L)血清肿瘤坏死因子-$\alpha$含量相比损伤非治疗组(573.8 ± 178.2 ng/L; 552.3 ± 169.6 ng/L)明显降低($P = 0.018$, $P = 0.013$)；血清中性粒细胞弹性蛋白酶含量在爆炸后24 h (62.6 ± 19.5 ng/mL)相比损伤非治疗组(97.6 ± 36.2 ng/mL)明显降低($P = 0.007$)。高剂量乌司他丁治疗组血清肿瘤坏死因子-$\alpha$在爆炸后12 h (356.1 ± 131.0 ng/L)较爆炸损伤组(573.8 ± 178.2 ng/L)明显降低($P = 0.004$)，血清中性粒细胞弹性蛋白酶含量在爆炸后24 h (72.3 ± 21.3 ng/mL)明显降低($P = 0.036$)。结论：高剂量乌司他丁治疗能够下调血清肿瘤坏死因子-$\alpha$及中性粒细胞弹性蛋白酶的表达水平，有助于急性肺损伤的治疗。**

## 关键词

**乌司他丁，急性肺损伤，肿瘤坏死因子-$\alpha$，中性粒细胞弹性蛋白酶**

# Investigation to the Expression of Cytokines in Serum of Rabbits with Acute Lung Injury Interposed by Different Doses Ulinastatin in Underwater Explosion

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## Abstract

**Objective: The concentrations of TNF-$\alpha$ and NE were detected for elucidating the change concentration in sera of rabbits with acute lung injury interposed by different doses Ulinastatin therapy in underwater explosion. Methods: Underwater explosion appliance was applied to cause acute lung injury of rabbits. The experimental rabbits were classified into control group, injury group, Ulinastatin therapy group (low dose group and high dose therapy group), respectively. Ulinastatin was given to therapy group after the blast instantly. The concentrations of TNF-$\alpha$ and NE in sera were detected at 4, 12 and 24 hours after bursting. Results: Serum TNF-$\alpha$ content in low dose Ulinastatin group was lower at 12 h (400.6 ± 79.0 ng/L) and 24 h (356.4 ± 181.2 ng/L) after explosion than that in group injury group (573.8 ± 178.2 ng/L, 552.3 ± 169.6 ng/L) ($P$ = 0.018, $P$ = 0.013). And similarly, serum NE content was significantly lower at 24 h after explosion (62.6 ± 19.5 ng/mL) than that in injury group (97.6 ± 36.2 ng/mL) ($P$ = 0.007). On the other hand, serum TNF-$\alpha$ in high dose group was significantly decreased ($P$ = 0.004) at 12 h after explosion (356.1 ± 131.0 ng/L) compared with that in injury group (573.8 ± 178.2 ng/L). Serum NE content was also significantly lower at 24 h after explosion (72.3 ± 21.3 ng/mL) than that in injury group ($P$ = 0.036). Conclusion: The level of TNF-$\alpha$ and NE in sera was more efficiently decreased by using different doses Ulinastatin therapy, which may be conducive to elucidating the mechanism of therapy to ALI.**

## Keywords

**Ulinastatin, Acute Lung Injury, Tumor Necrosis Factor-Alpha, Neutrophil Elastase**



## 1. 引言

急性肺损伤(acute lung injury, ALI)是由脓毒血症、肺炎、创伤等引起的肺损伤，伴随大量炎症细胞的浸润，中性粒细胞是其中最为活跃的一个群体[1]。炎症发生后可激活肺泡巨噬细胞分泌大量的促炎因子，这些细胞因子进一步趋化中性粒细胞向肺组织和毛细血管内聚集，引起更多炎性递质释放，形成级联放大效应造成肺实质的损伤[2]。在众多炎症介质中，作为中性粒细胞，嗜天青颗粒脱颗粒释放出的主要丝氨酸蛋白酶之一的中性粒细胞弹性蛋白酶(neutrophil elastase, NE)不仅参与细胞内外病原菌清除的非氧途径[3]；还可以和 NADPH 氧化酶系统结合而消化被吞噬的兵员微生物；另外还可以通过细胞外抗微生物途径，即胞外中性粒细胞捕捉器(neutrophil extracellular traps, NET)介导肺血管周围炎症的发生、发展[4]。肿瘤坏死因子-$\alpha$ (tumor necrosis factor-alpha, TNF-$\alpha$)以膜结合型和可溶性分子形式存在，二者均具有生物学活性[5]，TNF-$\alpha$ 在炎症性疾病中具有多种功能[6]，炎症发生时，TNF-$\alpha$ 蛋白及其可溶性受体水平均增加，且和炎症严重程度呈正相关[7] [8]。基于此，我们在复制动物急性肺损伤模型的基础上，以乌司他丁进行干预治疗，检测血清中 TNF-$\alpha$ 和 NE 的浓度，以揭示乌司他丁干预对 ALI 细胞因子表达的影响及其可能的机制。

## 2. 材料与方法

### 2.1. 实验动物及分组

选取体重 1.8 ± 0.2 kg SPF 级实验兔，实验前 3 天进行适应性喂养，密切观察，实验当天禁。将实验

动物随机分为对照组(n = 10)、损伤非治疗组(n = 10)、乌司他丁治疗组(2.5 万 U/kg、10 万 U/kg) (高、低剂量组 n 均为 10)。

### 2.2. 仪器与试剂

血气分析仪(美国艾利尔 Epoc®)；DNM-9602 酶标分析仪(北京普朗)；−80℃冰箱(日本三洋)；TNF-$\alpha$ 检测试剂盒(上海江莱生物，批号 201602)；中性粒细胞弹性蛋白酶检测试剂盒(上海朗顿)；乌司他丁粉剂(广东天普生化医药)。

### 2.3. 实验方法

#### 2.3.1. 实施水下爆炸

按照氯胺酮 40 mg/kg，氟哌利多 1.6 mg/kg 的剂量混合麻醉药，经臀部肌肉注射，麻醉时间 3~5 min。麻醉后，将家兔仰卧位固定在特制木板上，尽量完全剃除颈前部和胸部毛发，充分暴露胸部。用碘伏消毒家兔颈部，作 L 型切口，分别从左颈总动脉和右颈外静脉放置留置针。手术完成后，再次检查留置针的固定情况，具体做法参照文献[9]，以 1.0 g 太安炸药作为爆炸源，设置爆心距为 1.8 m。

#### 2.3.2. 采集动脉血气标本

在抽取动脉血之前，将 5 ml 注射器用肝素溶液(用生理盐水配成 1000 U/ml)浸润管壁，并保留适量肝素溶液使其充满注射器头部以排空空气。在爆炸后 4 h，12 h，24 h 分别通过左颈总动脉抽取 2 ml 动脉血。原则上借助动脉血压足以推动针芯使动脉血自动进入含肝素液的注射器内，然后立即将注射器插入橡胶塞以隔绝空气。

#### 2.3.3. 收集血清

分别在爆炸致伤后不同时间点经左颈总动脉置管抽取动脉血 5 ml，离心并收集血清，标记后冻存在−80℃冰箱备用。

### 2.4. 统计学处理

数据在 SPSS 17.0 统计软件上处理，$P$ 小于 0.05 视为有统计学差异。

## 3. 结果

TNF-$\alpha$，NE 均为在肺部炎症反应中发挥重要作用的炎症因子，对于炎症的发生发展具有重要的作用[10] [11]。乌司他丁治疗 ALI 后的血清细胞因子检测结果显示：2.5 万 U/kg 乌司他丁干预后血清 TNF-$\alpha$ 在爆炸后 12 h、24 h 相比爆炸损伤组降低($P$ = 0.018，$P$ = 0.013) (见表 1)；NE 在 24 小时降低($P$ = 0.007) (见表 2)。10 万 U/kg 乌司他丁干预后血清 TNF-$\alpha$ 在 12 小时时降低($P$ = 0.004) (见表 1)；而 NE 在 24 小时

**Table 1.** Comparison of TNF-$\alpha$ levels in serum in different groups of rabbits ( $\bar{X} \pm S$ , ng/L)
**表 1.** 不同组实验兔血清 TNF-$\alpha$ 含量比较( $\bar{X} \pm S$ , ng/L)

| Group | 4 h | t | $P$ | 12 h | t | $P$ | 24 h | t | $P$ |
|---|---|---|---|---|---|---|---|---|---|
| Control | 549.5 ± 230.2 | | | 598.8 ± 227.7 | | | 672.4 ± 157.9 | | |
| Injury | 538.2 ± 201.4 | | | 573.8 ± 178.2 | | | 552.3 ± 169.6 | | |
| Low dose | 386.9 ± 109.2 | 2.088 | 0.051 | 400.6 ± 79.0 | 2.809 | 0.012 | 356.1 ± 131.0 | 2.895 | 0.010 |
| High dose | 433.5 ± 247.3 | 1.038 | 0.313 | 356.4 ± 181.2 | 2.705 | 0.015 | 422.3 ± 208.8 | 1.528 | 0.144 |

**Table 2.** Comparison of NE levels in serum in different groups of rabbits ( $\bar{X} \pm S$ , ng/mL)
**表 2.** 不同组实验兔血清 NE 含量比较( $\bar{X} \pm S$ , ng/mL)

| Group | 4 h | t | *P* | 12 h | t | *P* | 24 h | t | *P* |
|---|---|---|---|---|---|---|---|---|---|
| Control | 95.5 ± 23.2 | | | 106.0 ± 20.9 | | | 99.9 ± 23.2 | | |
| Injury | 83.4 ± 25.8 | | | 88.2 ± 23.7 | | | 97.6 ± 36.2 | | |
| Low dose | 69.4 ± 27.4 | 1.178 | 0.254 | 67.7 ± 23.2 | 1.953 | 0.067 | 62.6 ± 19.5 | 2.693 | 0.015 |
| High dose | 84.1 ± 18.5 | 0.070 | 0.945 | 74.9 ± 24.5 | 1.232 | 0.234 | 72.3 ± 21.3 | 1.904 | 0.073 |

降低($P$ = 0.036) (见表 2)。以上结果提示，细胞因子在 ALI 炎症损伤中发挥着重要作用，乌司他丁可能通过下调相关炎症因子的表达进而治疗疾病。

## 4. 讨论

ALI 是由脓毒血症、肺炎、创伤等引起的肺急性损伤，伴随大量炎症细胞的浸润，在 ALI 的治疗上，可以通过抑制炎症细胞分泌的细胞因子达到治疗的目的。ALI 造成肺内中性粒细胞、巨噬细胞的富集，炎症因子的释放导致内皮细胞受损，毛细血管通透性增加、出血、水肿等病理现象[12] [13]，NE、TNF-$\alpha$ 等炎症因子均属此列。NE 正常情况下储存在中性粒细胞嗜天青颗粒内[14]，在遇到适当刺激的情况下，NE 被嗜天青颗粒释放出来，转位至细胞核内，参与组蛋白的降解、促进染色质的解凝、参与血栓的形成[15]。NE 的释放能够刺激包括弹性蛋白、胶原、纤维连接蛋白、层粘蛋白在内的 ECM 成分进行有害的组织重塑，因此其被认为是最具破坏力的酶类之一，能够分解几乎所有细胞外基质和许多重要的血浆蛋白[16]。NE 还可以诱导 IL-8、TNF-$\alpha$ 等趋化因子的表达和释放[17]。这些炎性介质又能反过来趋化和激活中性粒细胞释放更多的 NE，由此构成级联放大的炎症反应，形成继发性肺损伤。NE 酶活性的抑制不仅能够减轻疾病的进展而且能够逆转疾病的进程[18]。我们在前期水下爆炸致兔 ALI 模型建立的基础上[9]，以不同剂量乌司他丁治疗 ALI，结果显示低剂量乌司他丁干预时，TNF-$\alpha$ 治疗 12 h、24 h 后表达下调，NE 含量在 24 小时降低。高剂量乌司他丁干预 TNF-$\alpha$ 在治疗 12 小时后下降，而 NE 在干预 24 小时后下降，且肺水肿、肺出血等症状明显缓解。这一结果可能对以 NE、TNF-$\alpha$ 等炎症因子为治疗靶点进行炎症性疾病的治疗提供一个新的视角。

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