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发酵、梨支原体感染小鼠小肠黏膜和胰腺组织细胞电镜观察

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摘　要：目的　探讨发酵支原体（*Mycoplasma fermentans*，*Mf*）和梨支原体（*Mycoplasma pirum*，*Mpi*）感染致死小鼠小肠黏膜和胰腺组织细胞超微结构病理改变。**方法**　35只清洁级ICR小鼠，隔日腹腔注射环磷酰胺（50 mg/kg·d）5次制成免疫抑制模型。分为*Mf*、*Mpi*感染组（$n=12$）和生理盐水（NS）对照组（$n=11$），分别腹腔注射0.3 mL标准菌株*Mf*、*Mpi*（$6\times10^{8-9}$/mL）和无菌生理盐水，计算各组死亡率，取小鼠血液进行支原体再培养以及感染12 h、6 d后取小肠黏膜和胰腺组织超微结构观察。**结果**　免疫抑制小鼠*Mf*或*Mpi*感染6 d后，全部死亡，支原体再培养阳性。电镜观察显示：*Mf*或*Mpi*感染12 h后，小肠黏膜和胰腺组织细胞即表现超微结构病理改变，感染6 d后，小肠黏膜组织细胞显著水肿，肿胀，线粒体空泡，胰腺细胞线粒体空泡，酶原颗粒变小，细胞变性、坏死，与NS组比较差异显著，且胰腺组织细胞病理改变比小肠黏膜明显。**结论**　发酵支原体或梨支原体感染小鼠12 h后，小肠黏膜和胰腺组织细胞即表现超微结构病理改变，6 d后出现细胞水肿、变性及坏死。且胰腺组织细胞病理改变比小肠黏膜组织细胞更明显。

关键词：发酵支原体；梨支原体；电镜；胰腺；小肠黏膜

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Electron microscope observation on mouse intestinal mucosa and pancreatic tissue cells induced by *Mycoplasma fermentans* and *Mycoplasma pirum*

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ABSTRACT：To investigate the *Mycoplasma fermentans*（*Mf*）and *Mycoplasma pirum*（*Mpi*）infection and the ultrastructure pathology change of pancreas tissue in mouse intestine，35 ICR mice were made for immunosuppression models by intraperitoneal injection of cyclophosphamide for five times every other day and mice were divided into *Mf* infection group（n＝12），*Mpi* infection group（$n=12$），and the NS control group（n＝11）. They were respectively received 0.3 mL（$6\times10^{8-9}$/mL）standard strains of *Mf* and *Mpi* and the sterile saline by intraperitoneal injection to evaluate the mortality rates in each group. The serum samples from each group of mice were taken for re-cultivation of the *Mycoplasma*. And the intestinal mucosa and pancreatic tissue of the mice were subjected to ultrastructural observation by electron microscope after 12 hours and 6 days infection，respectively. All of the mice in *Mf* and *Mpi* infection groups were dead after infection for 6 days，and the *Mycoplasma* re-cultivation was positive. Ultrastructural observations indicate that after 12 hours of *Mf* or *Mpi* infection，intestinal mucosa and pancreatic tissue cell had ultrastructurally pathological changes. In the 6^{th} day after infection，significantly edema，swelling of mitochondria，vacuoles，mitochondria vacuoles of pancreatic cells，zymogen granules degeneration，and necrosis could be observed in the small intestinal mucosa cells，which showed significant difference compared with those in the NS group. And the pancreas tissue pathological change is more serious. The experiment results indicate that intestinal mucosa and pancreatic tissue cell change the ultrastructural pathology afte 12 hours of *Mf* or *Mpi* infection. After 6 days，edema，denaturation and necrosis could be observed in the cells，and the pathological changes in pancreas tissue are more significant than that in small-intestine mucosa in mice

KEY WORDS：*Mycoplasma fermentans*；*Mycoplasma pirum*；electron microscope；pancreas；intestinal mucosa

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发酵支原体(*Mycoplasma fermentans*, *Mf*)、梨支原体(*Mycoplasma pirum*, *Mpi*)因在HIV感染者和AIDS患者体内成功分离而被称为艾滋病相关支原体[1-3]。国内外研究表明,AIDS患者协同感染此两种支原体加速器官衰竭的发生是协同致死的重要原因[4]。多器官功能不全综合征(multiple organ dysfunction syndrome,MODS)是AIDS患者快速致死常见结局[3-4]。研究提示,动物细菌等病原体感染之后,机体小肠粘膜和胰腺组织病理改变常发生在MODS的早期,启动细胞因子效应,称"细胞因子风暴"。我们曾报道此两种支原体感染免疫缺陷大鼠,在5~7_d之内全部死亡,并在电镜下证实心、肺、肝、肾组织多器官损害[5],但未见免疫缺陷动物协同感染*Mf*和*Mpi*小肠粘膜和胰腺组织病理改变相应报道。为此,本实验取*Mf*和*Mpi*分别感染免疫缺陷小鼠,电镜动态观察小鼠肠粘膜和胰腺组织病理改变,试图为*Mf*和*Mpi*协同HIV感染致死的防治提供实验依据。

1 材料与方法

1.1 材料

1.1.1 标准菌株及培养基等的制备 见参考文献[6-7]。

1.1.2 实验动物 35只6周龄清洁级ICR雌性小鼠(16~20 g/只)由温州医学院动物中心提供。

1.1.3 试剂 环磷酰胺(0.2 g/瓶)购自江苏恒瑞医药股份公司(批号10052621)。

1.2 方法

1.2.1 菌液的制备 将冻干标准菌株复苏传代至对数期,离心后沉淀物菌落计数备用[6-7]。

1.2.2 实验动物分组及动物模型的制备 35只ICR小鼠(清洁级),隔日腹腔注射环磷酰胺50 mg/(kg·d),5次制成免疫缺陷模型。分为*Mf*、*Mpi*感染组(n=12)和*NS*对照组(n=11),分别腹腔注射0.3 mL标准菌株*Mf*、*Mpi*($6\times10^{8-9}$/mL)和无菌生理盐水,*Mf*和*Mpi*致死性感染组分别腹腔注射对数生长期*Mf*、*MPi* 0.6 mL ($3\times2\times10^{8-9}$);

1.2.3 血清的采集及支原体再培养 分别取实验组和对照组血清接种于SP4液体培养基中进行再培养。阳性,接种于固体培养基(肝消化汤琼脂)中孵育48 h,出现典型"油煎蛋"样菌落即可确认为阳性。

1.2.4 电镜标本的采集 各取感染后(12 h)、感染死亡期(6 d)和NS一只小鼠小肠粘膜和胰腺组织电镜液固定,电镜下待观察。

1.2.5 超薄切片电镜观察 取实验组死亡小鼠小肠和胰腺组织0.5 cm ×0.5 cm ×0.5 cm,包埋,铜网制作见参考文献[6-7],电镜下观察,拍片。

1.3 死亡率比较

观察各组存活数,采用SPSS18.0进行统计分析比较生存率差异。

2 结 果

2.1 死亡率比较

*Mf*和*Mpi*免疫抑制感染组在感染后死亡率均为100%(11/11),与NS组0%(0/11)比较,差异有统计学意义($P<0.01$),*Mf*与*Mpi*感染组无显著差异($P>0.05$),感染各组死亡率结果见表1。

表1 各组死亡率比较

Tab. 1 Comparison of mortality rate in each experimental group

Group	n	No. death/ mortality rate	*P* value
Mf infection	11	11/100%	$P>0.05$**
Mpi infection	11	11/100%	
NS control	11	0/0%	0.000*
Total	33	22/ 33, 66.7 %	

*: The *Mf* or *Mpi* infection group was significantly different with NS control group in immortality rate, $P<0.01$; **: No significant difference between the *Mf* and *Mpi* infection group was found, $P>0.05$.

2.2 支原体分离再培养结果

NS对照组未见支原体菌落,感染组支原体分离培养均见"油煎蛋"样阳性菌落,为*Mf*或*Mpi*再培养阳性。

2.3 超微结构观察

2.3.1 肠黏膜组织细胞电镜下观察 免疫抑制组小鼠*Mf*或*Mpi*感染后12 h,细胞核固缩变形,线粒体空泡,细胞核轻度固缩,肠腺上皮细胞粘液增多和聚集;感染6 d,肠黏膜组织细胞显著水肿、肿胀,线粒体空泡,与NS组差异显著(图1)。

2.3.2 胰腺组织细胞电镜下观察 免疫抑制组小鼠*Mf*或*Mpi*感染12 h后,胰腺组织胞核固缩,线粒体肿胀,并空泡化,酶原颗粒有轻度褪色性改变,直至6 d死亡后解剖,电镜下见细胞肿涨,胞核固缩,线粒体空泡,酶原颗粒变小,着色变浅。细胞肿胀、变性、坏死,与NS组结构差异显著(图2)。

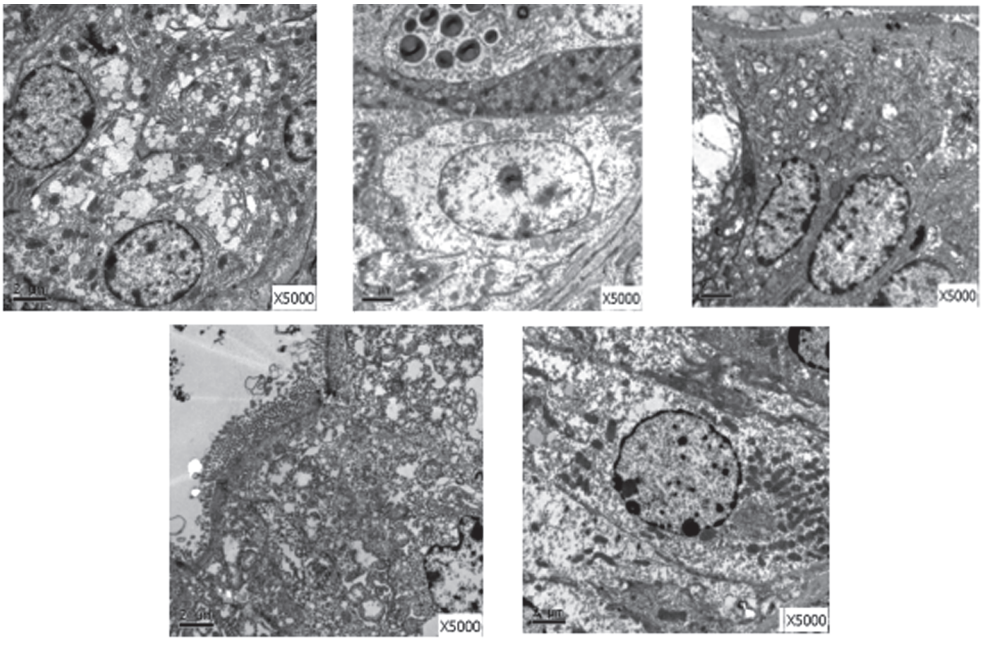

图 1-1　小肠黏膜上皮细胞电镜检查

Fig. 1-1　Electron microscopy observation on epithelial cells of small intestinal mucosa

After 12 hours of *Mf* infection, the nuclear showed mild pyknosis; mild expansion could be observed in rough endoplasmic reticulum, and the mucus particles of midgut gland epithelial cells became fusion and aggregation.

图 1-2　*Mf* 感染 6 d

Fig. 1-2　Six days after *Mf* infection

Edema was observed in small intestinal mucosal cell nuclear, organelles reduced, rough endoplasmic reticulum expanded, mitochondria vacuoles appeared, and the edema, degeneration and necrosis could be observed in cellular.

图 1-3　*Mpi* 感染 12 h

Fig. 1-3　Twelve hours after *Mpi* infection

The nuclear showed pyknosis and deformation, and the mitochondria vacuoles appeared.

图 1-4　*Mpi* 感染 6 d

Fig. 1-4　Six days after *Mpi* infection

Small intestinal mucosa microvilli showed edema and falling off; rough endoplasmic reticulum expanding; mitochondria vacuoles appeared, and the edema could be observed in cellular.

图 1-5　NS 对照组小肠黏膜组织

Fig. 1-5　Small intestinal mucosa cells in NS control group

No corresponding pathological change was observed.

以上结果提示 *Mf* 或 *Mpi* 感染后 12 h 小肠黏膜、组织和胰腺组织细胞即表现超微结构病理改变，直至 6 d 死亡更为严重，胰腺病变程度比小肠黏膜更明显。

3　讨　论

临床观察和实验研究表明，在重症感染过程中，两个或两个以上器官发生程序性功能衰竭，其中，肠道和胰腺可能是 MODS 发病的敏感器官，发生在 MODS 的早期，并在 MODS 发生、发展有着重要作用[8-9]。创伤、感染、休克等均可不同程度导致肠粘膜屏障损伤，诱发肠源性脓毒症和 MODS[8]。胰腺炎初始阶段是胰腺消化酶的激活和腺泡细胞损伤，二期的特点是胰腺炎症反应和不同程度的腺泡细胞坏死涉及约 12～72 h，最终，第三阶段所涉及进一步发展的胰腺损伤和 MODS[9]。细胞因子网络和免疫功能紊乱很可能是胰腺炎从局部病变迅速，这些炎性递质逸入血液循环激活了机体其他炎症细胞，释放大量炎性递质。这些炎性递质激发单核巨噬细胞分泌 TNFα，IL1、6、8 等炎性介质所产生的一系列连锁反应促成全身炎症反应综合征(SIRS)，迅速发展为 MODS 。MODS 是继持续放大和自我破坏的炎症过程，表现为播散性炎症细胞活化，MODS 是 SIRS 进行性加重的最终后果[10-11]。我们的实验曾证实大鼠腹腔接种发酵支原体和梨支原体后，免疫缺陷大鼠感染全部死亡(100%，7/7)，与非免疫缺陷组致死率差异显著[5]。电镜下发现免疫缺陷发酵支原体和梨支原体感染大鼠心、肝、肺、肾和

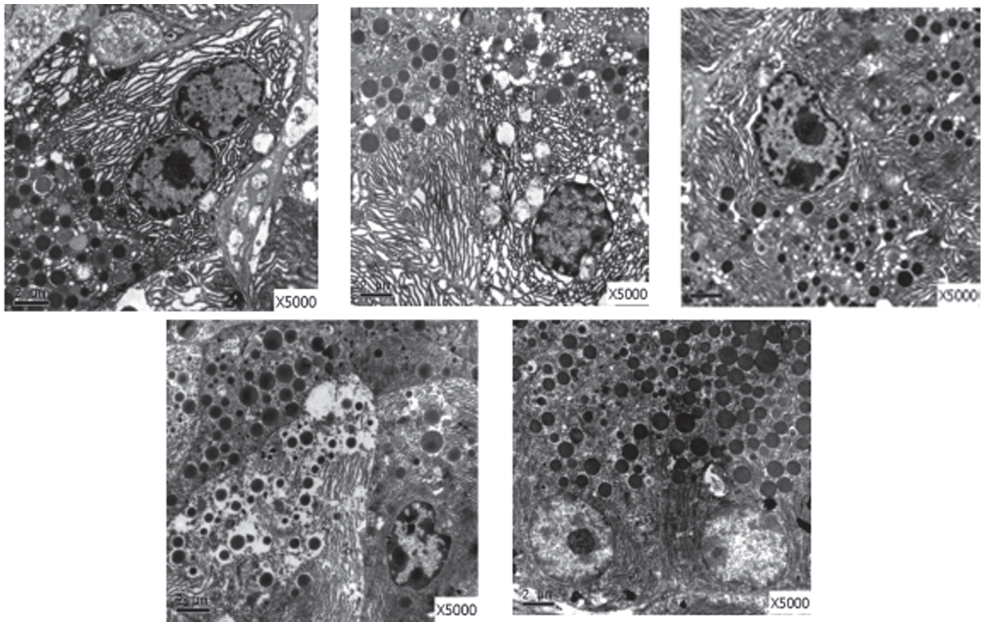

图 2-1 胰腺组织细胞电镜观察

Fig. 2-1 Electron microscopy observation on cells of pancreatic tissue

After 12 hours of *Mf* infection, the nuclear showed pyknosis; gap of nuclear membrane expanded; rough endoplasmic reticulum expanded; mitochondria vacuoles appeared; some zymogen granules showed coloration and were faded.

图 2-2 *Mf* 感染 6 d

Fig. 2-2 Six days after *Mf* infection

The nuclear showed pyknosis; gap of nuclear membrane expanded; rough endoplasmic reticulum expanded; mitochondria vacuoles appeared; zymogen granules showed coloration and were faded; the edema, degeneration and necrosis could be observed in cellular.

图 2-3 Mpi 感染 12 h

Fig. 2-3 Twelve hours after *Mpi* infection

The nuclear showed pyknosis; rough endoplasmic reticulum expanded slightly; the mitochondria vacuoles appeared; the edema and degeneration could be observed in cellular.

图 2-4 *Mpi* 感染 6 d

Fig. 2-4 Six days after *Mpi* infection

Mitochondria vacuoles appeared; zymogen granules became smaller; coloration became lighter; the edema, degeneration and necrosis could be observed in cellular.

图 2-5 NS 对照组胰腺组织细胞电镜观察

Fig. 2-5 No pathological change in NS control group

脑组织等重要器官严重损伤,在大鼠的心、脑、肺和肝组织细胞中发现相应三层膜结构的支原体,提示发酵和梨支原体感染可导致大鼠 MODS 的发生[5]。我们的实验还提示克林霉素等抗生素对免疫缺陷大鼠 *Mf* 和 *Mpi* 感染具保护作用[12]。进一步证实抗生素可为 *Mf* 和 *Mpi* 感染导致 MODS 提供保护。本研究采用腹腔注射 *Mf* 和 *Mpi*,分别感染免疫抑制小鼠,制备多器官功能衰竭综合征(*MODS*)模型,观察 *Mf* 和 *Mp* 感染 *i* 致死免疫抑制小鼠第一时段(感染后 12 h)和感染死亡期小肠黏膜和胰腺超微结构改变,并与免疫抑制非感染小鼠的小肠黏膜和胰腺超微结构改变予以比较。结果提示免疫抑制组小鼠 *Mf* 或 *Mpi* 感染后 12 h,小肠黏膜和胰腺细胞核固缩变形,线粒体空泡,且感染 6 d,小肠和胰腺细胞,线粒体空泡,显著水肿,细胞肿胀、变性和坏死,与 NS 组差异显著(图 1,2)。提示 Mf 或 Mpi 感染后 12 h,小肠黏膜和胰腺组织细胞超微结构病理改变,且胰腺的病变严重于小肠黏膜。我们的动物实验结果与 Yi-min Zhu 等报道[9]5 例感染 MODS 死亡的孩子经解剖均证实胰腺坏死和多脏器损害(肾上腺,肝,肺,心,脾),合并感染的胰腺坏死导致脓毒症,多器官功能障碍综合征,且这 5 例 MODS 孩子死亡相继发生在入院后 6 d 之后的 2 h 之内的临床观察相吻合。此实验结果将为 *Mf* 和 *Mpi* 协同 HIV 感染致死的防治提供实验依据。

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