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常咯啉、利多卡因、胺碘酮对培养大鼠搏动心肌细胞超微结构的影响

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提要　常咯啉(CRL)100 μg/ml使培养大鼠心肌细胞搏动消失，结构受损，电镜下肌原纤维排列不齐，胞浆内出现空泡及致密染色颗粒。利多卡因（Lid）1000和胺碘酮(Ami) 50 μg/ml时，也出现上述变化。当CRL为25，Lid为250及Ami 6.25 μg/ml时，光镜下细胞形态无改变而超微结构已有轻微损害。结果表明，CRL及Lid在临床上应用达到有效血药浓度时，对心肌细胞无毒性作用。

关键词　常咯啉；利多卡因；胺碘酮；培养大鼠搏动心肌细胞；电子显微镜检查

前文[1]报道了在超过有效血药浓度10-30倍的常咯啉(changrolin，CRL)作用下，培养的大鼠心肌细胞的搏动和形态有明显损伤、致死，伴有谷草转氨酶(AST)释放增高。并与利多卡因(lidocaine，Lid)，胺碘酮(amiodarone，Ami)进行了比较。本文旨在观察上述药物在不同浓度时对培养大鼠搏动心肌细胞的超微结构影响，以进一步了解这类药物对心肌细胞损害的精确部位。

Materials and methods

出生1-3 d的Sprague-Dawley大鼠心脏，用0.1%胰蛋白酶溶液分次消化细胞，细胞制备同前文[2]。生长液用含20%小牛血清的MEM Eagle's液[3]。CRL注射液(50 mg/ml)由上海天丰制药厂生产。Lid及Ami均系上海海普药厂制剂。

光学显微镜检查　消化所得细胞悬液，分装为每瓶含2.5×10^6细胞，加生长液至4 ml，置37℃培育48 h，心肌细胞以100 bpm规则地成片搏动，然后弃液，再分别加入含不同浓度CRL，Lid或Ami的生长液各4 ml/瓶，分组情况及毒性观察指标同前文[1]。

透射电镜检查　上述各组加入不同种类及浓度的药物后24 h，观察细胞搏动及光镜下形态改变，随即将无Ca^{2+}、Mg^{2+} Hank's液洗涤细胞2次，再用2.5%戊二醛固定3-4 h。然后将贴壁细胞自培养瓶壁刮下，置4℃离心($800\times g$) 20 min，电镜制样方法同前[3]，在日立H-300电子显微镜下观察。

Results

细胞搏动及病变　心肌细胞培养48 h，分别加入CRL 100，Lid 1000和Ami 50 μg/ml，24 h后细胞均停止搏动，并出现细胞团缩、空泡形成及胞浆内致密染色颗粒等病变。对照组细胞搏动良好而无上述改变。Ami 25 μg/ml时，约70%心肌细胞停止搏动，细胞内有少量空泡及黑色颗粒。CRL 50 μg/ml，Lid 500 μg/ml及Ami 12.5 μg/ml时，心肌细胞均搏动，但部分细胞内仍见少量空泡及黑色颗粒。CRL 25 μg/ml，Lid 250 μg/ml，Ami 6.25 μg/ml时，光镜下细胞无病变，且心肌细胞均成片搏动，与在24孔塑料板中所见相似。

超微结构改变　培养3 d的大鼠心肌细胞，在电镜下，细胞的超微结构均属正常，细胞核呈椭圆形，肌原纤维排列规则，有清楚的

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肌小节和Z线，闰盘清晰可见。线粒体众多，嵴密集，细胞质内糖原颗粒丰富，桥粒样结构清晰（Fig 1-A，Plate 1，以下各图同）。心肌细胞培养2 d加CRL 100 μg/ml 24 h后，肌原纤维有断裂，Z线不清晰，线粒体肿胀，膜边界不清，部分嵴排列紊乱，胞浆内出现很多空泡及致密染色颗粒（Fig 1-B）与光镜下所见空泡及黑色颗粒相符合，有些细胞核明显畸变且缩小，核染色质分布在核膜周围（Fig 1-B，右下角）。CRL 25 μg/ml时，细胞核形态正常，线粒体嵴已接近正常，仍可见胞浆内小空泡及少量致密染色颗粒（Fig 1-C）。CRL 12.5 μg/ml时，心肌超微结构已基本正常。

培养心肌细胞在Lid 1000 μg/ml作用下，细胞超微结构变化与CRL 100 μg/ml时基本相似，肌原纤维断裂，排列乱，Z线不清晰，线粒体膜不完整，嵴紊乱不清，胞浆内有空泡及致密染色颗粒，细胞核有畸变（Fig 1-D）。Lid 250 μg/ml时，线粒体众多，嵴清晰，闰盘有轻度解离（Fig 1-E）。Lid 125 μg/ml时，肌原纤维、Z线排列整齐、清晰，基本与对照细胞相似。

在培养心肌细胞中加入Ami 50 μg/ml，细胞的细胞器改变基本相似于CRL 100 μg/ml的变化，肌纤维排列紊乱，线粒体边缘模糊，嵴不清，胞浆内亦有空泡及致密染色颗粒，核畸变（Fig 1-F）。Ami 6.25 μg/ml时，虽细胞搏动良好，肌原纤维排列整齐，粗面内质网稍扩张，胞浆内有小空泡而未见染色颗粒（Fig 1-G）。

每种样品各重复3次实验，每次每个样品制4-5个切片观察。

Discussion

CRL，Lid及Ami 3种药物在致心肌细胞明显毒性反应时，除肌原纤维破坏，核改变外，胞浆内出现多数空泡及致密染色颗粒。这类改变是否系药物致细胞毒性的一种反应，尚不明了。

前文[1]中，我们在光镜下观察CRL、Lid及Ami对培养心肌细胞的毒性反应，发现CRL 25，Lid 250及Ami 6.25 μg/ml对心肌细胞的形态无明显改变，而本文在相同的剂量作用下，发现对心肌细胞的超微结构已稍有损害。因此，在评定药物对培养心肌细胞的毒性反应时，以电镜下的变化作为评定依据较为严格，它要求临床使用的血药浓度较低，以增加用药的安全性。

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Cytotoxic effects of changrolin, lidocaine and amiodarone on ultrastructure of cultured rat beating cardiac myocytes

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ABSTRACT Ultrastructural and morphological alterations of cultured rat beating cardiac myocytes treated with changrolin (CRL), lidocaine (Lid), and amiodarone (Ami) were studied. After the cultures were treated with CRL 100 μg/ml for 24 h, the beating of the myocytes stopped, the configuration and fine structure were destroyed, while the nuclei showed pyknotic deformation and reduced in size. The membrane and structures of mitochondria were disrupted and myofibrills fragmented and disrupted. In addition, a lot of vacuoles with characteristic dense particles were found in the cytoplasm. Similar alterations were seen when Lid 1000 μg/ml and Ami 50 μg/ml were added to the cultures. Normal beating networks of myocytes were examined under inverted microscopy after the cultured cells were treated with CRL 25 μg/ml, Lid 250 μg/ml or Ami 6.25 μg/ml. The ultrastructure of some regions of the myocytes showed very slight damage. The results indicated that the dosage of CRL and Lid generally used in anti-arrhythmic therapy basically exerted no harm to myocytes. However, caution should be taken when Ami was given intravenously, since its effective serum concentration was close to the dosage which could cause slight damage to the ultrastructure of cultured cells.

KEY WORDS changrolin; lidocaine; amiodarone; cultured rat beating heart cells; electron microscopy

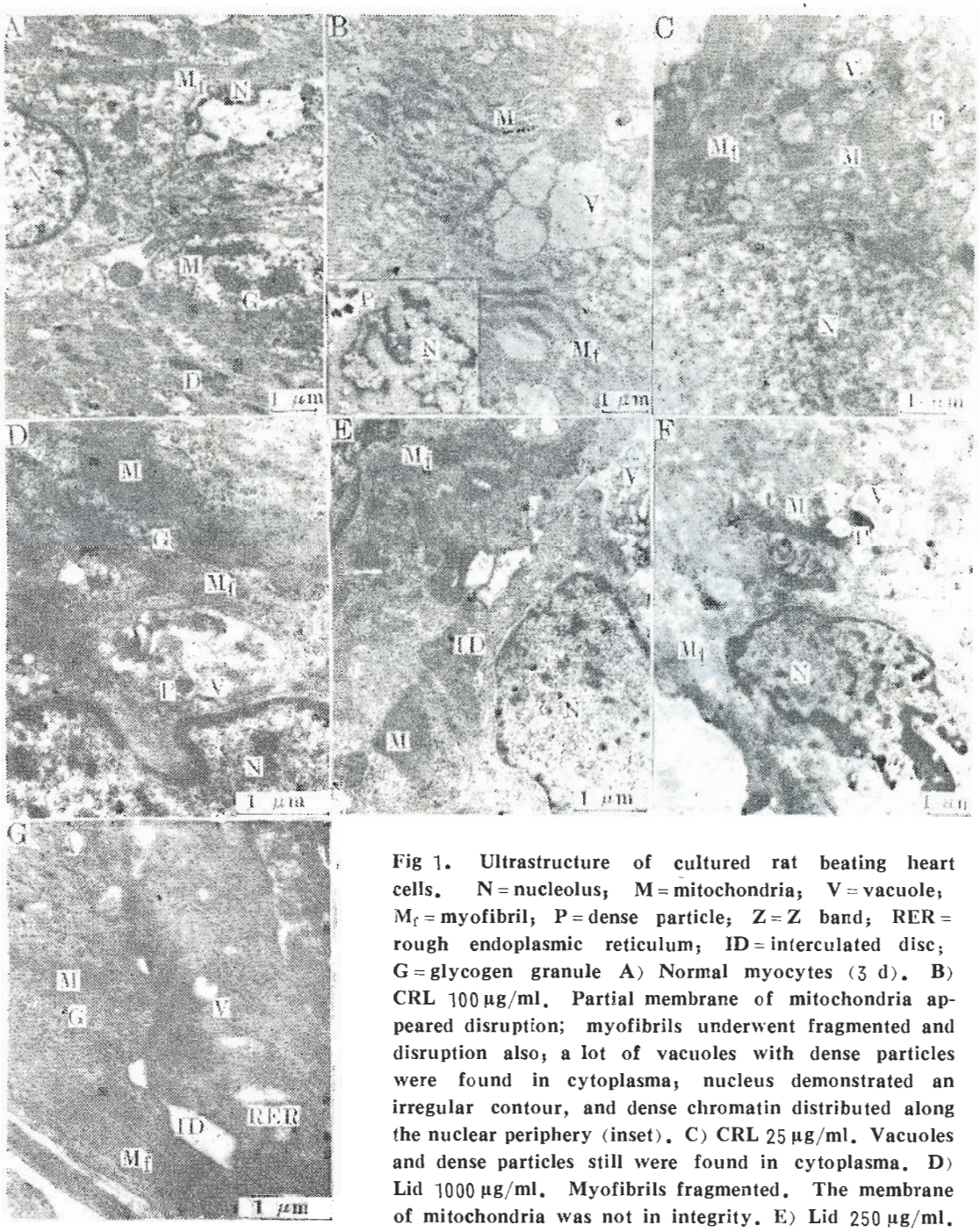

Fig 1. Ultrastructure of cultured rat beating heart cells. N = nucleolus; M = mitochondria; V = vacuole; M_f = myofibril; P = dense particle; Z = Z band; RER = rough endoplasmic reticulum; ID = interculated disc; G = glycogen granule A) Normal myocytes (3 d). B) CRL 100 μg/ml. Partial membrane of mitochondria appeared disruption; myofibrils underwent fragmented and disruption also; a lot of vacuoles with dense particles were found in cytoplasma; nucleus demonstrated an irregular contour, and dense chromatin distributed along the nuclear periphery (inset). C) CRL 25 μg/ml. Vacuoles and dense particles still were found in cytoplasma. D) Lid 1000 μg/ml. Myofibrils fragmented. The membrane of mitochondria was not in integrity. E) Lid 250 μg/ml. Nearly no vascuole or particle was found, interculated disc demonstrated slightly disintegrated. F) Ami 50 μg/ml. Myocyte damage was similar as that in Lid 1000 μg/ml. G) Ami 6.25 μg/ml. Rough endoplasmic reticulum increased in size, and a few of vacuoles still could be shown while no particle was demonstrated.

(See p 47)