新型铂配合物 Mor-platin 导致细胞凋亡及抑制细胞迁移

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摘要 探讨了新型铂配合物Mor-platin对人肺癌细胞(H1975)的抗癌效果以及对细胞迁移和侵袭的影响. 运用噻唑蓝法和活性氧检测试剂盒, 比较Mor-platin与经典抗癌药物顺铂影响H1975细胞存活率和活性氧产生的不同; 利用细胞周期与细胞凋亡检测试剂盒, 研究Mor-platin和顺铂对细胞凋亡与细胞周期的影响; Western blot法检测Mor-platin对caspase相关蛋白caspase 3/8/9/脱氧核糖核酸修复酶(PARP)蛋白水平和凋亡相关蛋白B-淋巴瘤-2基因相关蛋白(Bax)/B-淋巴瘤-2基因(Bcl-2)比值的改变; 最后使用划痕实验和transwell装置研究Mor-platin和顺铂对H1975细胞迁移和侵袭的影响. 实验结果显示, Mor-platin与顺铂相比, 在更低的浓度下即能杀死H1975细胞. 另外, Mor-platin比顺铂更易产生细胞内活性氧, 使细胞阻滞于S期, 激活caspase相关蛋白caspase 3/8/9/PARP, 增加Bax/Bcl-2比值, 最终导致细胞凋亡. 通过划痕与transwell实验还发现, Mor-platin可以抑制肿瘤细胞迁移和侵袭, 而顺铂没有这种现象. 综上所述, Mor-platin具有更好的抗肺癌细胞能力, 以及独特的抑制细胞迁移能力, 为铂类化合物的开发和肿瘤药物的研究提供了新思路.

关键词 顺铂, 抗癌药物, 活性氧, 细胞周期, 细胞迁移

铂类抗癌药物顺铂(cisplatin)作为临床上主要的化疗药物, 在治疗睾丸癌、卵巢癌、肺癌、膀胱癌和头颈癌中得到广泛应用^[1~4]. 传统铂类配合物进入体内后引起的肿瘤细胞凋亡或坏死的机制包括: 与细胞核脱氧核糖核酸(deoxyribonucleic acid, DNA)结合, 形成顺铂-DNA加合物, 阻碍细胞周期^[5,6], 产生或释放活性氧(reactive oxygen species, ROS)^[7,8]. 以铂类药物为基础的化疗虽然在治疗某些肿瘤方面具有很好的效果, 但是产生的耐药性和不可避免的不良反应, 使铂类药物的广泛应用受到限制^[9,10]. 为了克服耐药性并提高抗癌效果, 有必要继续研究新的铂类

配合物.

继顺铂问世以来, 研究者合成了数千种类似的铂类配合物, 约有30种进入临床试验阶段, 但在临床上应用较为普遍的只有3种, 分别是卡铂、奈达铂和奥沙利铂. 由于它们与顺铂不管是构效关系还是抗肿瘤机制方面都非常相似, 并与顺铂存在着交叉耐药性, 因此限制了其在临床上的广泛应用. 近年来, 基于对铂类配合物抗癌机制的深入研究和对经典铂类配合物存在的一些缺点的了解, 研究者大胆设计出了一些不同于原有构效关系的非经典铂类配合物, 最具代表性的有: 具有空间位阻的铂配合物^[11~13]、反

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式铂配合物^[14~16]、多核铂配合物^[17,18]、四价铂配合物^[19]。这些非经典铂类配合物可在一定程度上避免产生耐药性,或改善水溶性实现口服给药。虽然目前在铂类配合物研究当中取得了很大进展,但是仍有很多工作要做。

相比于正常细胞,癌症细胞中产生活性氧(ROS)的量更多,这些增加的活性氧无论对激活癌细胞还是对癌症细胞的生长都至关重要^[20,21]。然而,当外源性物质使细胞产生过多的活性氧时,易使细胞产生氧化应激。氧化应激会通过一系列途径,如内质网损伤、线粒体级联反应,最终导致细胞凋亡^[22,23]。因此,通过调节癌症细胞的活性氧水平是杀死肿瘤细胞的一种有效途径。众所周知,肿瘤转移是癌症致死的主要原因,90%的肿瘤患者死亡都可以归因于肿瘤转移,因此临床肿瘤治疗有必要避免肿瘤转移。肿瘤转移是癌症治疗的一大挑战,一旦发生肿瘤转移,就会产生差的预后或严重的不良反应。新兴抗肿瘤药物只有在清楚肿瘤转移情况下才能得到发展与应用^[24,25]。然而,顺铂在肿瘤治疗中可以通过减少药物内吞量、增强DNA修复和肿瘤转移导致药物耐药性,从而限制了其在临床上的应用^[26]。Shen等人^[27]发现,纺锤体和动粒相关蛋白-1基因(spindle and kinetochore associated complex, *SKA1*)可以调节非小细胞肺癌的肿瘤转移和耐药性,Li等人^[28]也发现,miR-27a可以通过靶向到人肺腺癌细胞中的Raf激酶抑制蛋白(raf kinase inhibitor protein, *PKIP*)调节顺铂耐药性和肿瘤转移,说明药物耐药性常和肿瘤转移同时发生。文献报道,顺铂只有和其他抗癌药物联合使用才能抑制肿瘤转移,提高患者生存率^[29~31]。因此研发能够减少肿瘤细胞转移的铂类配合物势在必行。本课题组^[32]成功合成一种新型铂类配合物Mor-platin (complex 6, 图1(a)), Mor-platin铂配合物是亲脂阳离子化合物,它不同于传统铂类配合物的平面结构,具有一定的空间结构,可以避免在与DNA结合前与含硫大分子结合,从而增加细胞内药物浓度,增强抗癌效果^[33,34]。前期研究结果显示, Mor-platin杀死肿瘤细胞的效果明显高于传统药物顺铂,但是对肿瘤细胞的其他生物学效应尚未进行研究。在本研究中,再次验证这种新型铂配合物Mor-platin对人肺癌细胞(H1975)的毒性明显大于顺铂,而且还发现细胞内的活性氧含量明显高于顺铂,并且激活了凋亡相关蛋白和caspase相关蛋白,使得细胞阻滞于S期,最终导

致细胞凋亡。更加重要的是,划痕实验显示Mor-platin能够阻止H1975细胞愈合,transwell实验发现, Mor-platin也能抑制H1975细胞迁移和侵袭,这些结果预示着Mor-platin将会成为一种不仅抗癌效果强,而且还能抑制肿瘤转移的抗肿瘤药物。

1 实验

1.1 试剂与仪器

人肺癌细胞H1975细胞系(中国科学院上海细胞库, 中国), 细胞培养基(dulbecco modified eagle medium, DMEM)培养基(Hyclone, 美国), 胎牛血清(fetal bovine serum, FBS) (Gibco, 美国), 胰酶(碧云天生物技术有限公司, 上海), 噻唑蓝(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide, MTT) (Sigma-Aldrich, 美国), 细胞周期与细胞凋亡检测试剂盒、活性氧检测试剂盒、BCA蛋白浓度测定试剂盒(碧云天生物技术有限公司), transwell小室(Corning, 美国), 羊抗鼠caspase 3、羊抗兔caspase 8、羊抗兔caspase 9、羊抗兔脱氧核糖核酸修复酶(poly (ADP-ribose) polymerase, PARP)、羊抗兔Bax (Bcl-2-associated X protein)、羊抗兔Bcl-2抗体(B-cell lymphoma 2) (Cell Signaling Technology, 美国), 羊抗鼠甘油醛-3-磷酸脱氢酶 (glyceral-dehyde-3-phosphate dehydrogenase, GAPDH)抗体(Santa Cruz, 美国), 辣根过氧化物酶(horseradish peroxidase, HRP)-山羊抗兔、山羊抗鼠二抗(碧云天生物技术有限公司)。

细胞培养箱(Thermo, 美国), 酶标仪(BioTek, 美国), 倒置荧光显微镜(Olympus, 日本), 流式细胞仪(BD, 美国)。

1.2 实验方法

(i) 细胞活力测试。选取对数生长期的H1975细胞, 用0.25%胰酶消化细胞, 制备单细胞悬液, 以每孔8000/100 L的细胞浓度接种于96孔板中, 将96孔板置于培养箱(37℃, 5% CO₂)中孵育24 h。待细胞完全贴壁后, 弃掉原培养基, 加入不同浓度的Mor-platin和顺铂, 以1%二甲基亚砜溶剂作为对照组, 同时设立不加细胞的空白对照组, 每个浓度有6个复孔。培养24 h后吸掉培养基, 加入MTT (100 μL, 0.5 mg mL⁻¹)继续孵育4 h, 吸掉上清, 加入100 μL二甲基亚砜避光振荡30 min, 以充分反应显色。用酶标仪测定波长在490 nm处的吸光度值。

(ii) 细胞凋亡实验. 实验操作参照细胞周期与细胞凋亡检测试剂盒的说明书. 选取对数增长期的H1975细胞, 用0.25%胰酶消化细胞, 以每孔 300000 mL^{-1} 的细胞浓度接种于6孔板中, 置于培养箱中孵育过夜, 加入不同浓度(0, 2, 4, 8, $10\text{ }\mu\text{g mL}^{-1}$)的Mor-platin和顺铂, 每个浓度有3个复孔. 药物处理24 h后, 将细胞培养液吸出至5 mL离心管中, 磷酸盐缓冲溶液(phosphate buffer saline, PBS)洗涤1次, 加入300 μL 胰酶消化液消化细胞, 放入培养箱消化2 min至细胞变圆后, 加入600 μL 培养基终止消化, 把细胞转移至先前的细胞培养液中, $1000\times g$ 离心5 min, 弃去上清, 预冷的PBS轻轻重悬细胞并计数. 取50~100000细胞, $1000\times g$ 离心5 min, 弃去上清, 加入195 L异硫氰酸荧光素(Annexin V-FITC)结合液轻轻重悬细胞, 加入5 μL Annexin V-FITC, 轻轻混匀, 最后加入10 μL 碘化丙啶混匀, 室温避光孵育30 min, 随即上流式细胞仪进行检测, 细胞凋亡率=细胞凋亡数/总细胞数 $\times 100\%$.

(iii) 活性氧检测. 细胞内的活性氧的产生用活性氧检测试剂盒, 是利用一种荧光探针2',7'-二氯荧光黄双乙酸盐(2,7-dichlorodihydrofluorescein diacetate, DCFH-DA), 其本身无荧光但可以自由穿过细胞膜, 进入细胞后, 会被细胞内的酯酶水解生成DCFH. 细胞内的活性氧可以把没有荧光的DCFH生成有荧光的DCF. 因此, 通过检测DCF的荧光来研究细胞内活性氧的水平. 各种不同浓度(0, 2, 4, $8\text{ }\mu\text{g mL}^{-1}$)的Mor-platin和顺铂处理H1975细胞24 h后, 收集细胞, 悬浮于用无血清培养基稀释的DCFH-DA (10 mol L^{-1})中, 于 37°C 避光孵育30 min. 为了使探针与细胞充分接触, 每隔3~5 min颠倒混匀一下. 弃掉培养基, 用无血清培养基洗3次, 最后用上流式细胞仪进行检测.

(iv) 细胞周期时相分析. 实验操作参照细胞周期与细胞凋亡检测试剂盒的说明书. 收集处理24 h的Mor-platin (0, 2, 4, $8\text{ }\mu\text{g mL}^{-1}$)组和对照组(1%二甲基亚砜溶剂)细胞, 利用预冷PBS洗2遍, 预冷的70%乙醇 4°C 固定过夜, 加入核糖核酸酶(ribonuclease, RNase)和碘化丙啶染液避光染色30 min, 用流式细胞仪作细胞周期分析.

(v) 凋亡相关蛋白表达量的检测. 不同浓度Mor-platin (0, 2, 4, 8, $10\text{ }\mu\text{g mL}^{-1}$)处理24 h后的细胞用放射免疫沉淀测定 (radio-immunoprecipitation assay, RIPA)裂解液(内含蛋白酶抑制剂、 $1\text{ mmol L}^{-1}\text{ NaVO}_3$ 、 $10\text{ mmol L}^{-1}\text{ NaF}$ 、 1 mmol L^{-1} 苯甲基磺酰氟

(phenylmethanesulfonyl fluoride, PMSF)和 1 mmol L^{-1} β -巯基乙醇)裂解细胞, BCA蛋白浓度测定试剂盒检测蛋白浓度, 蛋白样品加入 $6\times$ 上样缓冲液中 99°C 加热变性5 min, 用十二烷基硫酸钠-聚丙烯酰胺凝胶(sodium dodecyl sulfatepoly-acrylamide gel electrophoresis, SDS-PAGE)凝胶电泳分离蛋白, 然后转到硝基纤维素膜上, 5%牛奶蛋白封闭2 h, 一抗 4°C 孵育过夜, 三(羟甲基)氨基甲烷缓冲溶液和吐温20的混合溶液 (mixture of tris-buffered saline and tween 20, TBST)缓冲液洗3次, 每次5 min, 然后与接有辣根过氧化物酶的二抗室温孵育2 h, TBST缓冲液洗3次, 每次5 min, 用增强型化学发光液放射性自显影检测蛋白表达水平.

(vi) 细胞划痕实验和transwell实验. 细胞迁移首先通过划痕实验进行测定. H1975细胞以每孔 500000 mL^{-1} 的细胞浓度接种于6孔板中, 待细胞几乎长满时, 用1 mL枪头在细胞中间划一道痕迹, PBS洗掉漂浮细胞, 加入含有不同的Mor-platin和顺铂的小鼠 (*Mus musculus*) 胚胎成纤维细胞 (mouse embryonic fibroblast cells, NIH-3T3)细胞的上清液(诱导迁移), 在0和48 h时, 用倒置荧光显微镜进行拍照. Transwell实验步骤如下: Mor-platin (0, 1, $2\text{ }\mu\text{g mL}^{-1}$)处理24 h的细胞重悬于无血清培养基中($30000\text{ cells}/200\text{ }\mu\text{L}$), 接种到24-transwell上层小室中, 下层小室加入NIH-3T3细胞的上清液(诱导迁移), 小室之间避免产生气泡, 每个实验组设有3个重复. 侵袭实验除了上室膜要用基质胶(matrigel, $50\text{ }\mu\text{g mL}^{-1}$)处理外, 其他方法与迁移实验相同. 穿过膜的细胞用姬姆萨(Giemsa)染色液染色并计数.

(vii) 统计学处理. 计量资料以均数 $\pm$ 标准差($\bar{x}\pm\text{SD}$)表示, 采用SPSS 19.0统计学软件对实验数据进行处理, 组间比较采用单因素方差分析和Dunnett-t检验, $P<0.05$ 为差异有统计学意义.

2 结果

2.1 Mor-platin对细胞存活率的影响

采用MTT法考察不同浓度Mor-platin和顺铂对肺癌细胞存活率的影响, 如图1(b)所示, 在24 h内, 随着药物浓度的增加, 存活细胞数也随之下落, 具有剂量依赖性. 在相同浓度时, Mor-platin的存活细胞数少于顺铂, Mor-platin的半数抑制浓度(IC_{50})是 $14.41\text{ }\mu\text{g mL}^{-1}$, 而顺铂的 IC_{50} 是 $97.29\text{ }\mu\text{g mL}^{-1}$. 此结

图1 (网络版彩色) (a) Mor-platin的化学结构图; (b) Mor-platin和顺铂(Cisplatin)对H1975细胞的毒性实验

Figure 1 (Color online) (a) Chemical structure of Mor-platin; (b) cell viability of mor-platin and cisplatin on H1975 cells

果表明, Mor-platin较之顺铂具有更好的抗癌效果.

2.2 Mor-platin对细胞凋亡的影响

由MTT实验得知, Mor-platin能明显影响细胞存活率, 为了检测Mor-platin是否也能导致细胞凋亡, 用不同浓度(0, 2, 4, 10 $\mu\text{g mL}^{-1}$) Mor-platin和顺铂处理H1975细胞24 h后, 用流式细胞仪检测细胞凋亡情况, 结果如图2所示, 随着药物浓度的增加, 细胞凋亡率随之增加, 具有剂量依赖性. 在4 $\mu\text{g mL}^{-1}$ 以下浓度时,

图2 Mor-platin对H1975细胞凋亡的影响. 结果表示为 $\bar{x} \pm \text{SD}$, 3次重复, **: $P < 0.01$, 表示Mor-platin与顺铂对照组相比差异有统计学意义

Figure 2 Cell apoptosis of H1975 cells by Mor-platin. Results are expressed as $\bar{x} \pm \text{SD}$ from three independent experiments, **: $P < 0.01$ compared to cisplatin control

Mor-platin和顺铂导致的细胞凋亡数很少, 两者之间比较没有统计学差异. 从8 $\mu\text{g mL}^{-1}$ 开始, Mor-platin比顺铂的凋亡数明显增加, $P < 0.01$, 差异有统计学意义, 说明Mor-platin比顺铂较易导致细胞凋亡.

2.3 Mor-platin增加H1975细胞活性氧的产生

活性氧是正常细胞的一种代谢产物, 但是过多的活性氧对细胞是有害的. 活性氧是凋亡途径中的一种重要因素, 增加活性氧的产生会改变线粒体的膜电位, 破坏呼吸链, 最终导致细胞凋亡^[35]. 由图3

图3 Mor-platin和顺铂对H1975细胞活性氧产生的影响. **: $P < 0.01$, 与对照组相比有统计学差异

Figure 3 ROS generation in H1975 cells by Mor-platin or cisplatin. **: $P < 0.01$, compared to control

可知, Mor-platin从 $2\ \mu\text{g mL}^{-1}$ 开始产生大量活性氧, 且随着药物浓度的增加, 活性氧的产生也随之增加, 但顺铂在 $2\sim 8\ \mu\text{g mL}^{-1}$ 浓度之间并未对细胞产生明显的活性氧, 由此得知, Mor-platin比顺铂更易产生活性氧, 因此猜想Mor-platin比顺铂毒性更强的原因有可能是产生较多的活性氧。

2.4 Mor-platin使H1975细胞阻滞于S期

细胞增殖是由细胞周期来实现的. 众所周知, 细胞周期由4个时期组成: G_1 期(DNA合成前期)、S期(DNA合成期)、 G_2 期(DNA合成后期, 有丝分裂前期)、M期(有丝分裂期). 细胞周期能否启动、完成, 受许多调控机制的影响, 细胞周期检测点是其中一种重要的机制, 在细胞周期检测点中最重要的两个检测点是 G_1/S 和 G_2/M 检测点^[36]. 当真核细胞DNA受损时, 会通过阻滞细胞周期在 G_1 , S和 G_2/M 期从而阻碍细胞进程. 细胞周期检测点被激活是为了使在细胞周期恢复之前, 细胞能够完成一些重要的功能, 如DNA修复^[37]. 抗肿瘤药可以使细胞周期阻滞在 G_0/G_1 , S或者 G_2/M 期, 导致细胞凋亡^[38]. 文献报道, 顺铂以及其他一些铂类化合物主要抑制细胞周期在S或 G_2/M 期. 为了检测Mor-platin是否也会影响细胞周期, H1975细胞在无或有Mor-platin ($2, 4, 8\ \mu\text{g mL}^{-1}$)时培养24 h, 用流式细胞仪对细胞周期时相进行分析, 结果表明, Mor-platin使 G_0/G_1 期细胞数减少, 而S期细胞数增加, M期细胞数几乎没有变化, 说明Mor-platin可以使H1975细胞阻滞于S期, 从而阻碍细胞分裂, 抑制细胞增殖(图4).

图4 Mor-platin对H1975细胞周期的影响. 结果表示为 $\bar{x} \pm \text{SD}$, 3次重复, *: $P < 0.05$, **: $P < 0.01$, 与对照组相比有统计学差异

Figure 4 Effect of Mor-platin on cell cycle of H1975. Data represent $\bar{x} \pm \text{SD}$ from three independent experiments. *: $P < 0.05$, **: $P < 0.01$ compared to control

2.5 Western blot检测caspase相关蛋白以及细胞凋亡相关蛋白的表达

Caspase称为半胱氨酸天门冬氨酸酶, 在介导细胞凋亡的过程中起着非常重要的作用^[39]. 为了阐述Mor-platin导致细胞凋亡的机制, 利用western blot检测caspase 3/8/9和caspase 3的胞浆底物PARP蛋白表达水平, 说明Mor-platin是否通过激活caspase相关蛋白, 从而导致细胞凋亡^[40]. 另外, 促凋亡蛋白(Bax)和抗凋亡蛋白(Bcl-2)的比值被报道与肿瘤细胞的凋亡相关^[41]. Western blot结果显示, Mor-platin处理24 h后的细胞caspase 3/8/9/PARP, Bcl-2蛋白水平降低, 而Bax蛋白表达上调, Bax/Bcl-2比值升高, 因此, 可以推断, Mor-platin导致细胞凋亡是通过激活caspase 3/9和增加Bax/Bcl-2比值实现的(图5).

2.6 Mor-platin抑制细胞迁移和侵袭

大部分癌症患者死亡不是因为肿瘤本身, 而是由于肿瘤转移. 肿瘤转移时, 肿瘤细胞从原发部位侵入淋巴管或血管, 在远处继续增殖、生长, 形成转移瘤^[42]. 因此, 本实验还研究合成的药物Mor-platin是否会影响肿瘤细胞的迁移和侵袭. 首先, 细胞划痕实验(图6(a))显示, $2\ \mu\text{g mL}^{-1}$ 的Mor-platin能够抑制H1975细胞迁移. 接下来利用transwell实验对划痕实验结果进行验证, 经 $0, 1, 2\ \mu\text{g mL}^{-1}$ Mor-platin处理24 h的H1975细胞重悬于无血清培养基中, 加入transwell上

图5 Western blot分析不同浓度Mor-platin对H1975细胞的caspase相关蛋白以及凋亡相关蛋白表达的影响

Figure 5 Western blot analysis of caspase-related protein and apoptosis-associated proteins in H1975 cells treated with different concentrations of Mor-platin

图 6 (网络版彩色) Mor-platin和顺铂对H1975 细胞迁移和侵袭的影响. (a) 细胞划痕实验; (b) Transwell实验: 不同浓度Mor-platin处理过的H1975 细胞分别加入到transwell上室, 下室加入NIH-3T3 上清液(迁移诱导剂), 放入 37℃、5% CO₂ 条件下孵育 24 h后, 用姬姆萨(Giemsa)染色液染色, 倒置荧光显微镜 10×视野下观察迁移到下室的细胞并拍照

Figure 6 (Color online) Migration and invasion of H1975 toward Mor-platin and cisplatin. (a) Cell wound-healing assay; (b) transwell assay: Mor-platin treated H1975 cells were seeded in the upper chamber, migration inducer (NIH-3T3 cell conditioned medium) was added into the lower chamber. After incubation at 37°C 5% CO₂ for 24 h, cells that migrated and invaded through the membrane were stained with Giemsa, imaged and counted in 10× field under inverted fluorescence microscope

室, 下室加入NIH3T3上清液, 诱导迁移. Transwell迁移实验结果显示, 2 µg mL⁻¹ Mor-platin使H1975细胞穿过transwell上室到达下室的细胞数与对照组相比明显减少(图6(b)), 而1 µg mL⁻¹ Mor-platin穿过的细胞数与对照组差不多, 侵袭实验结果与迁移类似, 但是2 µg mL⁻¹ Mor-platin使H1975细胞穿过transwell小室的细胞数比迁移实验更少. 综上所述, Mor-platin可以明显抑制肿瘤细胞迁移和侵袭, 而且侵袭效果更明显.

3 结果与讨论

通过在传统铂类配合物的结构基础上进行改造, 合成一种不同于原来构效关系的新型铂配合物Mor-platin^[32]. 非小细胞肺癌约占肺癌总数的85%, 是全

球癌症死亡的主要原因^[43], 提高非小细胞肺癌的治疗效果仍然是全世界的一大挑战, 因此选择非小细胞肺癌细胞系H1975细胞作为实验研究对象. 实验结果表明, 此配合物与传统抗肿瘤药物顺铂相比, 对H1975细胞的杀伤效果更强. 有文献报道, 顺铂导致的细胞凋亡跟活性氧的产生有密切关系^[44,45]. 为了研究Mor-platin是否和顺铂一样通过产生ROS导致细胞凋亡, 本研究检测了Mor-platin在H1975细胞内产生的活性氧量. 经研究发现, Mor-platin使H1975细胞产生的活性氧量明显多于顺铂, 由此可以认为Mor-platin和顺铂类似, 都是通过产生过多的活性氧, 对细胞造成氧化应激, 并且比顺铂效果更好. 由实验结果得知, Mor-platin在2 µg mL⁻¹时就可以产生活性

氧,而Mor-platin从4 $\mu\text{g mL}^{-1}$ 才开始导致细胞周期阻滞,从8 $\mu\text{g mL}^{-1}$ 开始导致细胞凋亡,说明Mor-platin首先使H1975细胞产生活性氧,然后才导致细胞周期阻滞,最终导致细胞凋亡.经研究还发现,Mor-platin可以激活caspase蛋白,使得caspase 3/8/9, PARP蛋白表达下降, Bax/Bcl-2比值升高,说明Mor-platin也可以通过激活caspase蛋白和凋亡相关蛋白导致细胞凋亡.肿瘤患者死亡大多是由于原位瘤转移到远处,尽管传统化疗药物的抗癌效果很强,但是由于会对正

常细胞造成毒性和肿瘤转移,使得应用受到一定限制.肿瘤转移是癌症治疗中的一大难题,发生转移意味着较差的预后或严重影响患者的生存. Transwell实验还发现新合成的药物Mor-platin能够抑制肿瘤细胞迁移和侵袭,这也许可以减少肿瘤并发症的产生,具有很好的临床意义. Mor-platin不仅对肿瘤细胞的杀伤效果强,而且还能抑制肿瘤细胞迁移和侵袭,减少并发症的发生,它将可能会成为一种很好的抗肿瘤药,但是对其生物学机制还需进一步的深入研究.

参考文献

- Rosenberg B, Vancamp L, Krigas T. Inhibition of cell division in escherichia coli by electrolysis products from a platinum electrode. *Nature*, 1965, 205: 698–699
- Kelland L. The resurgence of platinum-based cancer chemotherapy. *Nat Rev Cancer*, 2007, 7: 573–584
- Scagliotti G V, Parikh P, von Pawel J, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naïve patients with advanced-stage non-small-cell lung cancer. *J Clin Oncol*, 2008, 26: 3543–3551
- Bruijninx P C, Sadler P J. New trends for metal complexes with anticancer activity. *Curr Opin Chem Biol*, 2008, 12: 197–206
- Jordan P, Carmo-Fonseca M. Molecular mechanisms involved in cisplatin cytotoxicity. *Cell Mol Life Sci*, 2000, 57: 1229–1235
- Kikuchi Y. The mechanism of cisplatin-resistance in ovarian cancer. *Hum Cell*, 2001, 14: 115–133
- Li D W, Sun J Y, Wang K, et al. Attenuation of cisplatin-induced neurotoxicity by cyanidin, a natural inhibitor of ROS-mediated apoptosis in PC₁₂ Cells. *Cell Mol Neurobiol*, 2015, 35: 995–1001
- Jawaid P, Rehman M U, Zhao Q L, et al. Helium-based cold atmospheric plasma-induced reactive oxygen species-mediated apoptotic pathway attenuated by platinum nanoparticles. *J Cell Mol Med*, 2016, 20: 1737–1748
- Ratzon E, Najajreh Y, Salem R, et al. Platinum (IV)-fatty acid conjugates overcome inherently and acquired Cisplatin resistant cancer cell lines: An *in-vitro* study. *BMC Cancer*, 2016, 16: 140
- Eckstein N. Platinum resistance in breast and ovarian cancer cell lines. *J Exp Clin Cancer Res*, 2011, 30: 91
- Margiotta N, Natile G, Capitelli F, et al. Sterically hindered complexes of platinum(II) with planar heterocyclic nitrogen donors. A novel complex with 1-methyl-cytosine has a spectrum of activity different from cisplatin and is able of overcoming acquired cisplatin resistance. *J Inorg Biochem*, 2006, 100: 1849–1857
- Yu Y, Xie M J, Liu W P, et al. Synthesis, crystal structures and anticancer activity of Pt(II) complexes with two sterically hindered 2-methylpyridine groups as the carrier. *Asian J Chem*, 2010, 22: 3127–3134
- Holford J, Beale P J, Boxall F E, et al. Mechanisms of drug resistance to the platinum complex ZD0473 in ovarian cancer cell lines. *Eur J Cancer*, 2000, 36: 1984–1990
- Fabijanska M, Studzian K, Szmigiero L, et al. Trans-Platinum(II) complex of 3-aminoflavone-synthesis, X-ray crystal structure and biological activities *in vitro*. *Dalton Trans*, 2015, 44: 938–947
- Quiroga A G. Understanding trans platinum complexes as potential antitumor drugs beyond targeting DNA. *J Inorg Biochem*, 2012, 114: 106–112
- Coluccia M, Natile G. Trans-platinum complexes in cancer therapy. *Anticancer Agents Med Chem*, 2007, 7: 111–123
- Apps M, Wheate N. Synthesis and characterisation of sterically hindered pyridine based multinuclear platinum anticancer complexes and their cytotoxicity. *J Clin Oncol*, 2014, 10: 28
- Borowski S C, Schreiner S. Synthesis and reactivity of multinuclear platinum and palladium complexes. *Abstr Pap Am Chem S*, 2006, 128: 231
- Raynaud F I, Boxall F E, Goddard P, et al. Metabolism, protein binding and *in vivo* activity of the oral platinum drug JM216 and its biotransformation products. *Anticancer Res*, 1996, 16: 1857–1862
- He G D, He G, Zhou R, et al. Enhancement of cisplatin-induced colon cancer cells apoptosis by shikonin, a natural inducer of ROS *in vitro* and *in vivo*. *Biochem Biophys Res Commun*, 2016, 469: 1075–1082
- Li Y, Wang Z C, Qi Y L. Effects of radiation on apoptosis, ROS and cell cycle in breast cancer stem cells. *J Invest Med*, 2014, 62: S2

- 22 Fiskus W, Saba N, Shen M, et al. Auranofin induces lethal oxidative and endoplasmic reticulum stress and exerts potent preclinical activity against chronic lymphocytic leukemia. *Cancer Res*, 2014, 74: 2520–2532
- 23 Xiang T, Du L, Pham P, et al. Nelfinavir, an HIV protease inhibitor, induces apoptosis and cell cycle arrest in human cervical cancer cells via the ROS-dependent mitochondrial pathway. *Cancer Lett*, 2015, 364: 79–88
- 24 Nguyen D X, Bos P D, Massague J. Metastasis: From dissemination to organ-specific colonization. *Nat Rev Cancer*, 2009, 9: 274–284
- 25 Spano D, Heck C, De Antonellis P, et al. Molecular networks that regulate cancer metastasis. *Semin Cancer Biol*, 2012, 22: 234–249
- 26 Wang D, Lippard S J. Cellular processing of platinum anticancer drugs. *Nat Rev Drug Discov*, 2005, 4: 307–320
- 27 Shen L, Yang M, Lin Q, et al. SKA1 regulates the metastasis and cisplatin resistance of non-small cell lung cancer. *Oncol Rep*, 2016, 35: 2561–2568
- 28 Li J, Wang Y, Song Y, et al. MiR-27a regulates cisplatin resistance and metastasis by targeting RKIP in human lung adenocarcinoma cells. *Mol Cancer*, 2014, 13: 193
- 29 Zhang P, He J B, Ou L W, et al. Effects of suramin in combination with cisplatin on growth and metastasis of lung adenocarcinoma xenografts in mice (in Chinese). *Chin J Cancer*, 2006, 25: 409–413 [张平, 贺兼斌, 欧立文, 等. 苏拉明联合顺铂对肺腺癌小鼠移植瘤生长和转移的影响. *癌症*, 2006, 25: 409–413]
- 30 Matsumoto G, Omi Y, Lee U, et al. *NK4* gene therapy combined with cisplatin inhibits tumour growth and metastasis of squamous cell carcinoma. *Anticancer Res*, 2011, 31: 105–111
- 31 Lee S W, Kim S G, Park Y W, et al. Cisplatin and 4-hexylresorcinol synergise to decrease metastasis and increase survival rate in an oral mucosal melanoma xenograft model: A preliminary study. *Tumour Biol*, 2013, 34: 1595–1603
- 32 Guo Z, Tong W L, Chan M C. Luminescent oligo(ethylene glycol)-functionalized cyclometalated platinum(II) complexes: Cellular characterization and mitochondria-specific localization. *Chem Commun (Camb)*, 2014, 50: 1711–1714
- 33 Yu H, Gou S, Wang Z, et al. Toward overcoming cisplatin resistance via sterically hindered platinum(II) complexes. *Eur J Med Chem*, 2016, 114: 141–152
- 34 Wang Z M, Wu M, Gou S H. Toward a better understanding of the oxaliplatin mode of action upon the steric hindrance of 1,2-diaminocyclohexane and its analogue. *J Inorg Biochem*, 2016, 157: 1–7
- 35 Jezek P, Hlavata L. Mitochondria in homeostasis of reactive oxygen species in cell, tissues, and organism. *Inter J Biochem Cell Biol*, 2005, 37: 2478–2503
- 36 Rodriguez L, Ochoa B, Martinez M J. NF-Y and Sp1 are involved in transcriptional regulation of rat *SND p102* gene. *Biochem Biophysical Res Commun*, 2007, 356: 226–232
- 37 Pucci B, Kasten M, Giordano A. Cell cycle and apoptosis. *Neoplasia*, 2000, 2: 291–299
- 38 Kabir S R, Nabi M M, Haque A, et al. Pea lectin inhibits growth of Ehrlich ascites carcinoma cells by inducing apoptosis and G₂/M cell cycle arrest *in vivo* in mice. *Phytomedicine*, 2013, 20: 1288–1296
- 39 Thornberry N A. Caspases: Key mediators of apoptosis. *Chem Biol*, 1998, 5: R97–R103
- 40 Lin G J, Jiang G B, Xie Y Y, et al. Cytotoxicity, apoptosis, cell cycle arrest, reactive oxygen species, mitochondrial membrane potential, and Western blotting analysis of ruthenium(II) complexes. *J Biol Inorg Chem*, 2013, 18: 873–882
- 41 Wang H Q, Xu Y, Yan J, et al. Acteoside protects human neuroblastoma SH-SY5Y cells against beta-amyloid-induced cell injury. *Brain Res*, 2009, 1283: 139–147
- 42 Chiang A, Massague J. Molecular basis of metastasis reply. *N Eng J Med*, 2009, 359: 2814–2823
- 43 Jemal A, Center M M, DeSantis C, et al. Global patterns of cancer incidence and mortality rates and trends. *Cancer Epidemiol Biomarkers Prev*, 2010, 19: 1893–907
- 44 So H, Kim H, Lee J H, et al. Cisplatin cytotoxicity of auditory cells requires secretions of proinflammatory cytokines via activation of ERK and NF- κ B. *J Assoc Res Otolaryngol*, 2007, 8: 338–355
- 45 Rybak L P, Husain K, Whitworth C, et al. Dose dependent protection by lipoic acid against cisplatin-induced ototoxicity in rats: Antioxidant defense system. *Toxicol Sci*, 1999, 47: 195–202

Summary for “新型铂配合物 Mor-platin 导致细胞凋亡及抑制细胞迁移”

Mor-platin a new platinum complex that can induce apoptosis and inhibit migration

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Since cisplatin was discovered and applied clinically, people have synthesized thousands of platinum compound derivatives. These platinum derivatives are effective in chemotherapy for some tumors including testicular cancer, ovarian cancer, lung cancer, bladder cancer, head and neck cancer. All of these platinum complex are believed to exert their unparalleled anticancer potency by interacting with nuclear DNA, resulting in DNA adducts formation and consequently lead to cell apoptosis, cell cycle arrest and reactive oxygen species released. However, these platinum complex are severely limited by drug resistance and undesirable side effects arising from relatively low specificity and resultant high dosage requirement. To overcome the drug-resistance and improve therapeutic efficiency, it is necessary to synthesized new platinum complex. Our group successfully synthesized a new platinum complex Mor-platin, it can significantly damage more tumor cells than traditional drug cisplatin, while the other biological effects have not been studied.

This work aims to study the effect of Mor-platin on its anti-cancer ability, cell migration and cell invasion. In order to detect anti-cancer efficiency, MTT assay was used, and the results showed that IC_{50} of Mor-platin ($14.41 \mu\text{g mL}^{-1}$) was far less than IC_{50} of cisplatin ($97.29 \mu\text{g mL}^{-1}$), suggesting that Mor-platin have potentially higher tumor cytotoxicity than cisplatin. Next, we used reactive oxygen species assay kit, western blot analysis, apoptosis and cell cycle kit to assess whether Mor-platin can cause cell death by apoptosis, cell cycle, ROS, apoptosis-related protein and caspase protein. The results showed that Mor-platin can increase level of ROS, arrest cell cycle in S phase, increase ratio of Bax/Bcl-2 and activate caspase 3/8/9 resulting in cleaved PARP, ultimately lead to cell death.

The major threat of cancer is not from the primary tumor itself but from metastasis to other organs. To develop successful metastases, cancer cells need to disseminate to a distant organ, migrate out of the primary tumor, invade to blood or lymphatic vessels, finally seed, proliferate and colonize to form secondary tumors. Cell migration is a basic process within the cancer. Tumor metastasis is a major cause of cancer deaths, 90% of the cancer deaths can be attributed to the tumor metastasis, therefore it is necessary to avoid tumor metastasis on clinical cancer treatment. Only understanding the clear tumor metastasis for emerging antitumor drugs, they can be obtained to development and application. Current tumor metastasis experiments include transwell assay, wound-healing assay, microcarrier beads, three-dimensional assay and so on. Here, we used wound-healing assay and transwell assay to quantify the migration inhibitory activity of Mor-platin. Intriguingly, both wound-healing assay and transwell assay showed that Mor-platin can also inhibit cell migration and invasion, while cisplatin did not show any same effects. In summary, this new platinum complex Mor-platin not only have high anti-cancer efficiency but also inhibit migration, providing a novel strategy for the development of next generation platinum complex with optimal anti-tumor activities.

cisplatin, anti-cancer drugs, reactive oxygen species, cell cycle, cell migration

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