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医学生物物理

脂质体姜黄素联合索拉非尼对人肝癌Huh7细胞的抑制作用

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【摘 要】**目的：**探讨脂质体姜黄素联合索拉非尼对肝癌Huh7细胞的抑制作用。**方法：**采用脂质体包埋技术制备脂质体姜黄素，并通过Nano-ZS激光粒度测定仪测定其粒径大小及Zeta电位。分别用浓度为10 μmol/L脂质体姜黄素、0.1 μmol/L索拉非尼单独及联合处理人肝癌Huh7细胞，应用CCK8检测和EdU检测评价其对Huh7细胞增殖的影响，通过Transwell小室迁移实验检测其对Huh7细胞迁移能力的影响。**结果：**获得粒径为(118.7±13.6) nm、Zeta电位为(-9.8±1.1) mV的脂质体姜黄素，脂质体姜黄素单独使用可显著抑制Huh7细胞的增殖和迁移，脂质体姜黄素与索拉非尼联合使用可显著提高索拉非尼对Huh7细胞增殖与迁移的抑制能力。**结论：**脂质体姜黄素与索拉非尼联合使用可显著抑制肝癌Huh7细胞的增殖和迁移，是肝癌治疗的一种潜在策略。

【关键词】肝癌；脂质体姜黄素；索拉非尼；联合用药
【中图分类号】R392；R318 【文献标志码】A 【文章编号】1005-202X(2018)11-1324-05

Inhibitory effects of liposome curcumin combined with sorafenib on human hepatocellular carcinoma Huh7 cells

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Abstract: Objective To investigate the inhibition of the combination therapy of liposome curcumin and sorafenib on hepatocellular carcinoma Huh7 cells. **Methods** Liposome curcumin was prepared by liposome embedding technique, and its particle size and Zeta potential were determined by Nano-ZS laser particle size analyzer. Human hepatocellular carcinoma Huh7 cells were treated with liposome curcumin (10 μmol/L) and sorafenib (0.1 μmol/L) separately and jointly. The effects of liposome curcumin and sorafenib on the proliferation of Huh7 cells were evaluated with CCK8 assay and EdU assay, and their effects on the migration of Huh7 cells were detected by Transwell chamber migration assay. **Results** Liposome curcumin with a diameter of (118.7±13.6) nm and Zeta potential of (-9.8±1.1) mV was obtained. Using liposome curcumin alone significantly inhibited the proliferation and migration of Huh7 cells. The combined use of liposomal curcumin and sorafenib remarkably improved the inhibitory effects of sorafenib on the proliferation and migration of Huh7 cells. **Conclusion** The combined use of liposome curcumin and sorafenib which can inhibit the proliferation and migration of hepatoma Huh7 cells is a potential strategy for the treatment of hepatocellular carcinoma.

Keywords: liver cancer; liposomes curcumin; sorafenib; combination therapy

前 言

肝癌是全世界范围内最常见的恶性肿瘤之一，发病率居全球第三，死亡率居第五，具有高复发、高转移的特点，是人们生命健康的严重威胁[1-2]。随着手术治疗、放射介入治疗等技术手段的发展，少部分早期肝癌患者可以得到有效的治疗，而大部分中晚期肝癌患者的预后仍不理想[3]。目前，索拉非尼是唯一获美国食品药品监督管理局(Food and Drug Administration, FDA)批准的用于晚期肝癌治疗的靶向药物，可通过抑制多激酶信号通路抑制肿瘤的增殖和转移[4-5]。然而，临床研究表明，使用索拉非尼易产生耐药现象[6]。因此，开发具有抗肝癌作用的活性物质，通过联合用药增强索拉非尼治疗的敏感性，对提高中晚期肝癌患者的生存率，改善其生活质量具有重要意义。

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姜黄素(curcumin)是一种从姜科天南星科植物的根茎中分离纯化出的天然二酮类色素,具有抗氧化、抗炎、抗肿瘤等多种药理学活性[7-9]。近年,大量研究表明,姜黄素对肝癌、结肠癌、乳腺癌等多种恶性肿瘤的生长均有显著的抑制作用,并可以通过协同作用提高化学治疗、放射治疗等多种肿瘤治疗手段的治疗效果[10-13]。然而,水溶性差、体内半衰期短、生物利用度低等特点极大的限制了姜黄素在临床中的应用[14]。因此,本研究以纳米脂质体技术为依托,制备了以脂质体为载体的脂质体姜黄素,并评价了脂质体姜黄素及其与索拉非尼联用对肝癌Huh7细胞增殖能力和迁移能力的影响。

1 材料和方法

1.1 材料

姜黄素(分析纯,美国Sigma公司),卵磷脂、胆固醇、二氯甲烷(德国Merck公司);索拉非尼(德国Byer公司);DMEM高糖培养基、胰酶、新生牛血清、抗生素(美国Thermo公司);CCK8试剂盒(日本同仁公司);EdU荧光检测试剂盒(锐博生物科技公司);Transwell小室(德国Merck公司);CO_2培养箱(美国Thermo公司);Nano-ZS激光粒度测定仪(英国Malvern公司);荧光显微镜(德国Zeiss公司)。

1.2 方法

1.2.1 脂质体姜黄素制备及表征　将姜黄素、胆固醇、卵磷脂按10:35:200的比例混合,在40 ℃下完全溶解于二氯甲烷中。通过减压蒸馏去除二氯甲烷,并真空干燥过夜。将所获得的干燥样品溶解于pH值为6.5的PBS溶液中,在温度为50 ℃,剪切速率为3 000 rpm的条件下进行充分乳化,直至无可见颗粒物。乳化液分别通过粒径为0.45、0.22、0.10 μm的聚酯碳酸膜,于50 ℃高压热挤出,得到粒径一致的乳状液,过滤除菌后,于2~8 ℃条件下保存。

在洁净的聚苯丙乙烯色杯中,分别加入20 μL姜黄素脂质体乳状液和980 μL双蒸水,充分混匀,并排出气泡。采用Nano-ZS激光粒度测定仪测定脂质体的粒度和粒度分布。

将1 mL的姜黄素脂质体乳状液加入洁净的聚苯丙乙烯色U形管中,采用Nano-ZS激光粒度测定仪测定其Zeta电位。

1.2.2 细胞培养及功能学研究　Huh7细胞购自于中科院上海细胞库,使用DMEM高糖培养基,添加10%新生牛血清和100单位青霉素、链霉素,培养于37 ℃、5% CO_2培养箱中。实验分组为对照组、0.1 μmol/L索拉非尼组、10 μmol/L脂质体姜黄素组、0.1 μmol/L索拉非尼联合10 μmol/L脂质体姜黄素组。

通过CCK8实验检测细胞增殖能力。将对数生长期的Huh7细胞稀释为10 000个/mL的细胞悬液,接种于96孔板中,待细胞贴壁后,按分组加入药物进行处理,分别在处理12、24、36、48 h后加入CCK8试剂,反应2 h后,通过酶标仪检测吸光度值。

通过EdU实验检测细胞增殖能力。将对数生长期的Huh7细胞按照每孔20 000个铺于放有玻片的12孔板中,正常培养24 h,按照试剂盒说明书进行操作,经Edu标记、固定、Apollo染色、细胞核染色、封片等操作,最终在荧光显微镜下观察实验结果。

通过Transwell小室迁移实验检测细胞迁移能力。将对数生长期的Huh7细胞用无血清DMEM培养基稀释为100 000个/mL的细胞悬液接种于小室中,连续培养24 h。取出小室,用PBS轻轻洗涤,并浸泡于多聚甲醛溶液中固定30 min。再次取出小室,用PBS洗涤后,将小室放入结晶紫中染色30 min。洗去多余的结晶紫染液后,在显微镜下观察。

1.2.3 统计学分析　所有实验结果以均数±标准差的形式表示。利用GraphPad.Prism.v6.01软件进行数据分析。采用Student t检验进行实验组和对照组分析。当P值小于0.05时表示结果具有统计学意义。

2 结 果

2.1 姜黄素脂质体表征

依据中国药典2015相关规定,采用Nano-ZS激光粒度测定仪表征姜黄素脂质体颗粒粒径大小及Zeta电位。结果显示,姜黄素脂质体颗粒粒径均一,为(118.7±13.6) nm,Zeta电位为(-9.8±1.1) mV,呈负电。

2.2 姜黄素脂质体与索拉非尼单独及联合用药对Huh7细胞增殖的影响

本实验分别通过CCK8检测及EdU荧光检测评价了姜黄素脂质体与索拉非尼单独及联合用药对Huh7细胞增殖的影响。CCK8检测结果如图1所示,姜黄素脂质体和索拉非尼单独及联合使用均可抑制Huh7细胞增殖,两者联合使用的细胞增殖能力显著低于单独处理的细胞增殖能力,具有统计学差异(P<0.05)。如图2所示,EdU荧光检测结果表明,姜黄素脂质体和索拉非尼联合使用可显著抑制Huh7细胞增殖(P<0.05),其效果优于单独使用。

2.3 姜黄素脂质体与索拉非尼单独及联合用药对Huh7细胞迁移的影响

本实验通过Transwell小室实验检测了姜黄素脂质体与索拉非尼单独及联合用药对Huh7细胞迁移能

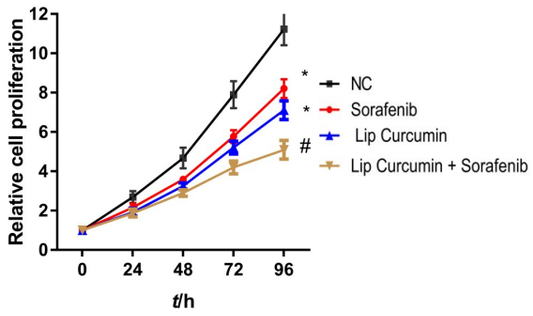


图1 CCK8检测索拉非尼、脂质体姜黄素单独和联合使用对Huh7细胞增殖能力的影响

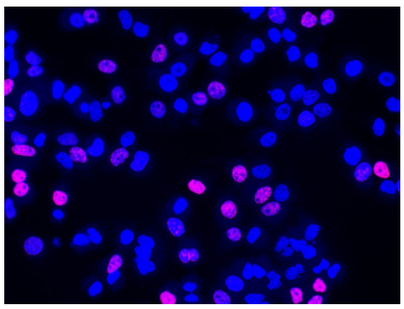
Fig.1 Effects of sorafenib, liposome curcumin and combination therapy of sorafenib and liposome curcumin on the proliferation of Huh7 cells detected by CCK8 assay

NC: Negative control; Compared with NC group, **P*<0.05; Compared with sorafenib group, #*P*<0.05

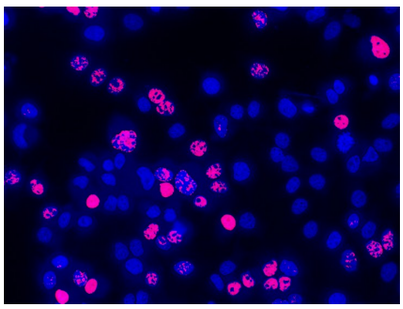
力的影响。实验结果如图3所示，姜黄素脂质体和索拉非尼单独使用均可抑制Huh7细胞迁移能力，两者联合使用的迁移抑制效果显著优于单独使用，具有统计学差异（*P*<0.05）。

3 讨 论

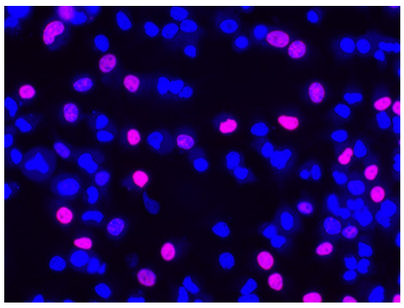
脂质体是一种“人工生物膜”，以磷脂和胆固醇为主要成分，在水溶液中自发形成具有双分子层结构的微型囊泡[15]。利用脂质体的双分子层结构特征，人们将其应用于药物包埋，将水溶性差的药物包裹在其囊泡内，输送到体内[16]。同时，脂质体包埋可以保护药物，提高药物的稳定性，并延长药物在体内滞留的时间，改善药物的作用效果[17-19]。因此，脂质体已经成为一种重要的纳米载药系统，广泛应用于



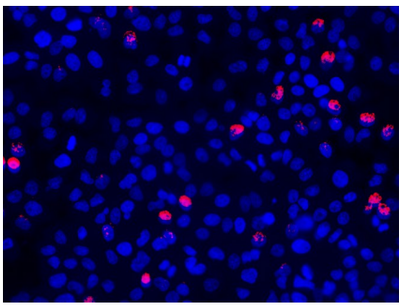
a: Proliferation of Huh7 cells from NC group obtained by EdU assay



b: Proliferation of Huh7 cells from sorafenib group obtained by EdU assay



c: Proliferation of Huh7 cells from liposome curcumin group obtained by EdU assay



d: Proliferation of Huh7 cells from combination therapy group obtained by EdU assay

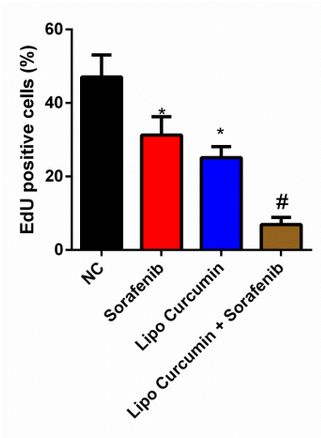


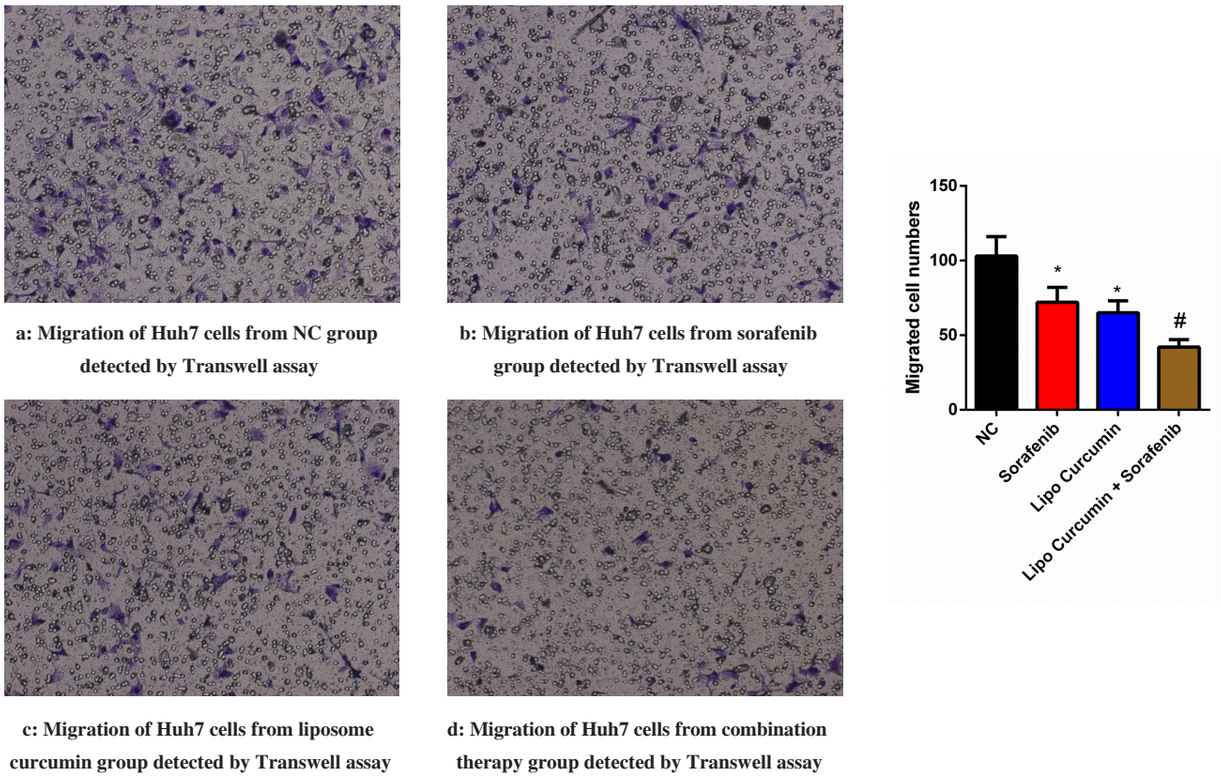
图2 EdU检测索拉非尼、脂质体姜黄素单独和联合使用对Huh7细胞增殖能力的影响（×200）

Fig.2 Effects of sorafenib, liposome curcumin and combination therapy of sorafenib and liposome curcumin on the proliferation of Huh7 cells detected by EdU assay (×200)

Compared with NC group, **P*<0.05; Compared with sorafenib group, #*P*<0.05

药物开发中。

近年来，姜黄素作为一种具有抑制肿瘤作用的生物活性物质获得了广泛的关注。大量研究表明，姜黄素可以从氧化应激、表观遗传、肿瘤微环境等多个层面发挥抗肿瘤作用[20-22]。Wang等[23]报道姜黄素可通过阻断microRNA-7641介导的促癌作用，抑制膀胱癌细胞的增殖和转移。Chatterjee等[24]的研究发现，姜黄素作为一种DNA甲基化抑制剂，通过降低基因启动子区域甲基化可促进p21基因表达，引起肿瘤细胞凋亡。然而，姜黄素水溶性差、生物利用度低的特点限制了其在临床上应用。为解决该问题，本研究通过脂质体药物载体制备技术，成功制备得粒径



a: Migration of Huh7 cells from NC group detected by Transwell assay

b: Migration of Huh7 cells from sorafenib group detected by Transwell assay

c: Migration of Huh7 cells from liposome curcumin group detected by Transwell assay

d: Migration of Huh7 cells from combination therapy group detected by Transwell assay

图3 Transwell小室检测索拉非尼、脂质体姜黄素单独和联合使用对Huh7细胞的迁移能力的影响（×100）

Fig.3 Effects of sorafenib, liposome curcumin and combination therapy of sorafenib liposome curcumin on the migration of Huh7 cells detected by Transwell assay (×100)

Compared with NC group, **P*<0.05; Compared with sorafenib group, #*P*<0.05

为（118.7±13.6）nm，Zeta电位为（-9.8±1.1）mV，均一、稳定的脂质体姜黄素。

在进一步的研究中，我们通过细胞功能学实验评价了脂质体姜黄素、脂质体姜黄素与索拉非尼联用对肝癌细胞的抑制作用。CCK8和EdU检测结果显示浓度为10 μmol/L脂质体姜黄素及0.1 μmol/L索拉非尼均能显著抑制肝癌Huh7细胞的体外增殖，而脂质体姜黄素与索拉非尼联用的增殖抑制效果显著优于单独使用。同时，transwell小室迁移实验结果表明，脂质体姜黄素或索拉非尼单独处理过的肝癌细胞的迁移能力下降，而两者联合处理的肝癌细胞的迁移能力显著低于单独处理后的细胞。

本研究探讨了脂质体姜黄素与索拉非尼联合用药对肝癌细胞增殖、迁移的影响，为中晚期肝癌患者的临床治疗提供了新机遇。

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