二氯乙酸钠对人骨肉瘤 MG63 细胞增殖、凋亡和迁移的影响

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摘　要　目的:观察二氯乙酸钠(sodium dichloroacetate,DCA－Na)对人骨肉瘤 MG63 细胞增殖、凋亡和迁移的影响。**方法:**将对数生长期的 MG63 细胞随机分为对照组和低、中、高浓度 DCA－Na 组,对照组不加 DCA－Na,低、中、高 DCA－Na 组加入 DCA－Na(终浓度分别为 50 μg·mL^{-1}、100 μg·mL^{-1}、200 μg·mL^{-1}),并设 1 个只加等量培养基不加细胞和 DCA－Na 的空白组。分别在干预 24 h、48 h、72 h 后,采用 Cell Counting Kit－8 细胞活性检测试剂盒分光光度法检测 MG63 细胞增殖情况,测定光密度(optical density,OD),计算低、中、高 DCA－Na 组细胞增殖抑制率,细胞增殖抑制率＝[1－(DCA－Na 组 OD 值－空白组 OD 值)/(对照组 OD 值－空白组 OD 值)]×100%;分别采用 Caspase－3 活性检测试剂盒分光光度法和 Annexin V－FITC 细胞凋亡检测试剂盒流式细胞法检测 MG63 细胞 Caspase－3 酶活性情况和细胞凋亡情况;并在干预 48 h 后采用 Transwell 实验检测 MG63 细胞迁移情况。**结果:**①细胞增殖检测结果。干预后,低、中、高 DCA－Na 组 MG63 细胞增殖抑制明显,且随干预时间的延长,3 组细胞增殖抑制率均呈上升趋势。各时间点间 MG63 细胞增殖抑制率差异有统计学意义(F＝847.080,P＝0.000),存在时间效应;干预 24 h、48 h、72 h 后,低、中、高 DCA－Na 组 MG63 细胞增殖抑制率的组间差异均有统计学意义,且低 DCA－Na 组＜中 DCA－Na 组＜高 DCA－Na 组[(10.802±3.000)%,(18.792±2.261)%,(27.080±3.133)%,F＝2.795,P＝0.000;(13.098±1.299)%,(25.215±2.676)%,(44.382±2.397)%,F＝4.362,P＝0.000;(14.728±1.177)%,(35.297±4.757)%,(64.227±4.549)%,F＝5.896,P＝0.000];3 组间 MG63 细胞增殖抑制率总体比较,差异有统计学意义,存在分组效应(F＝296.412,P＝0.000);时间因素与分组因素之间存在交互效应(F＝75.678,P＝0.000)。②Caspase－3 酶活性检测结果。对照组 MG63 细胞 Caspase－3 酶活性无明显变化,干预后低、中、高 DCA－Na 组 MG63 细胞 Caspase－3 酶活性均呈上升趋势。各时间点间 MG63 细胞 Caspase－3 酶活性的差异有统计学意义(F＝1 480.792,P＝0.000),存在时间效应;干预 24 h、48 h、72 h 后,4 组间 MG63 细胞 Caspase－3 酶活性的差异均有统计学意义,且对照组＜低 DCA－Na 组＜中 DCA－Na 组＜高 DCA－Na 组(0.027±0.003,0.143±0.005,0.153±0.008,0.161±0.003,F＝2.320,P＝0.000;0.035±0.003,0.174±0.004,0.184±0.007,0.253±0.001,F＝1.014,P＝0.000;0.031±0.004,0.246±0.006,0.275±0.003,0.371±0.004,F＝1.000,P＝0.000);4 组间 MG63 细胞 Caspase－3 酶活性总体比较,差异有统计学意义,存在分组效应(F＝624.975,P＝0.000);时间因素与分组因素之间存在交互效应(F＝94.579,P＝0.000)。③流式细胞法细胞凋亡检测结果。对照组 MG63 细胞凋亡率无明显变化,干预后低、中、高浓度 DCA－Na 组 MG63 细胞凋亡率均呈上升趋势。各时间点间 MG63 细胞凋亡率的差异有统计学意义(F＝359.645,P＝0.000),存在时间效应;干预 24 h、48 h、72 h 后,各组间 MG63 细胞凋亡率的差异均有统计学意义,且对照组＜低 DCA－Na 组＜中 DCA－Na 组＜高 DCA－Na 组[(2.554±0.427)%,(10.708±2.012)%,(20.857±2.531)%,(27.312±2.140)%,F＝6.733,P＝0.000;(1.748±0.202)%,(18.604±2.721)%,(29.471±1.605)%,(36.873±2.734)%,F＝9.292,P＝0.000;(1.944±0.112)%,(24.071±3.921)%,(30.050±3.921)%,(38.211±1.721)%,F＝8.237,P＝0.000];4 组间 MG63 细胞凋亡率总体比较,差异有统计学意义,存在分组效应(F＝46.627,P＝0.000);时间因素与分组因素之间存在交互效应(F＝7.012,P＝0.000)。④细胞迁移检测结果。干预 48 h后,4 组迁移细胞数[显微镜每个视野下(×200)]的组间差异有统计学意义[(84.45±10.45)个,(74.56±9.45)个,(65.41±5.21)个,(40.21±4.52)个,F＝148.243,P＝0.000)];低、中、高 DCA－Na 组迁移细胞数均少于对照组(P＝0.017,P＝0.001,P＝0.000),且低 DCA－Na 组＞中 DCA－Na 组＞高 DCA－Na 组(P＝0.012,P＝0.001,P＝0.000)。**结论:**DCA－Na 可抑制人骨肉瘤 MG63 细胞的增殖,增强 MG63 细胞 Caspase－3 酶活性,诱导和促进 MG63 细胞凋亡,抑制 MG63 细胞的迁移;且浓度越高、干预时间越长,其影响越明显。

关键词　骨肉瘤;二氯乙酸盐;细胞增殖;细胞凋亡;细胞运动;半胱氨酸天冬氨酸蛋白酶 3;MG63 细胞株

Effect of sodium dichloroacetate on cell proliferation,apoptosis and migration in human MG63 osteosarcoma cells

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ABSTRACT　Objective: To explore the effect of sodium dichloroacetate (DCA－Na) on cell proliferation, apoptosis and migration in

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human MG63 osteosarcoma cells. **Methods**: The MG63 cells were randomly divided into control group, low – concentration DCA – Na group, middle – concentration DCA – Na group and high – concentration DCA – Na group in the logarithmic growth phase. No DCA – Na were placed in cells in control group, and DCA – Na with final concentration of 50, 100 and 200 μg/mL were placed in cells in low – , middle – and high – concentration DCA – Na group respectively, meanwhile, a blank group was established with same amount of culture medium and without cells and DCA – Na. At 24, 48 and 72 hours after the intervention, the MG63 cell proliferations were detected by using Cell Counting Kit – 8 spectrophotometry and the optical density (OD) were measured for calculating the cell proliferation inhibition rate ([1 – (ODDCA – Na group – ODblank group)/(ODcontrol group – ODblank group)] × 100%) of low – , middle – and high – concentration DCA – Na group respectively. Meanwhile, The Caspase – 3 enzymatic activity and apoptosis of MG63 cells were detected by using Caspase – 3 activity assay kit spectrophotometry and Annexin V – FITC apoptosis assays kit flow cytometry respectively. The MG63 cell migration was detected by using Transwell assay at 48 hours after the intervention. **Results**: The MG63 cell proliferations were obviously inhibited after intervention in low – , middle – and high – concentration DCA – Na group, and a time – dependent rising trend of cell proliferation inhibition rate was found in the 3 groups. There was statistical difference in the MG63 cell proliferation inhibition rate between different timepoints ($F = 847.080$, $P = 0.000$), in other words, there was time effect. There was statistical difference in the MG63 cell proliferation inhibition rate between low – , middle – and high – concentration DCA – Na group at 24, 48 and 72 hours after the intervention, and the MG63 cell proliferation inhibition rate was lower in the low – concentration DCA – Na group compared to middle – concentration DCA – Na group and was lower in the middle – concentration DCA – Na group compared to high – concentration DCA – Na group (10.802 +/– 3.000, 18.792 +/– 2.261, 27.080 +/– 3.133%, $F = 2.795$, $P = 0.000$; 13.098 +/– 1.299, 25.215 +/– 2.676, 44.382 +/– 2.397%, $F = 4.362$, $P = 0.000$; 14.728 +/– 1.177, 35.297 +/– 4.757, 64.227 +/– 4.549%, $F = 5.896$, $P = 0.000$). There was statistical difference in MG63 cell proliferation inhibition rate between the 3 groups in general, in other words, there was group effect ($F = 296.412$, $P = 0.000$). There was interaction between time factor and group factor ($F = 75.678$, $P = 0.000$). No significant change was found in Caspase – 3 enzymatic activity of MG63 cells in control group, while a rising trend of the Caspase – 3 enzymatic activity of MG63 cells was found in low – , middle – and high – concentration DCA – Na group after intervention. There was statistical difference in the Caspase – 3 enzymatic activity of MG63 cells between different timepoints ($F = 1\,480.792$, $P = 0.000$), in other words, there was time effect. There was statistical difference in the Caspase – 3 enzymatic activity of MG63 cells between the 4 groups at 24, 48 and 72 hours after the intervention, and the Caspase – 3 enzymatic activity of MG63 cells presented a low – to – high trend in control group and low – , middle – and high – concentration DCA – Na group in turn (0.027 +/– 0.003, 0.143 +/– 0.005, 0.153 +/– 0.008, 0.161 +/– 0.003, $F = 2.320$, $P = 0.000$; 0.035 +/– 0.003, 0.174 +/– 0.004, 0.184 +/– 0.007, 0.253 +/– 0.001, $F = 1.014$, $P = 0.000$; 0.031 +/– 0.004, 0.246 +/– 0.006, 0.275 +/– 0.003, 0.371 +/– 0.004, $F = 1.000$, $P = 0.000$). There was statistical difference in Caspase – 3 enzymatic activity of MG63 cells between the 4 groups in general, in other words, there was group effect ($F = 624.975$, $P = 0.000$). There was interaction between time factor and group factor ($F = 94.579$, $P = 0.000$). No significant change was found in MG63 cell apoptosis rate in control group, while a rising trend of MG63 cell apoptosis rate was found in low – , middle – and high – concentration DCA – Na group after intervention. There was statistical difference in the MG63 cell apoptosis rate between different timepoints ($F = 359.645$, $P = 0.000$), in other words, there was time effect. There was statistical difference in the MG63 cell apoptosis rate between the 4 groups at 24, 48 and 72 hours after the intervention, and the MG63 cell apoptosis rate presented a low – to – high trend in control group and low – , middle – and high – concentration DCA – Na group in turn (2.554 +/– 0.427, 10.708 +/– 2.012, 20.857 +/– 2.531, 27.312 +/– 2.140%, $F = 6.733$, $P = 0.000$; 1.748 +/– 0.202, 18.604 +/– 2.721, 29.471 +/– 1.605, 36.873 +/– 2.734%, $F = 9.292$, $P = 0.000$; 1.944 +/– 0.112, 24.071 +/– 3.921, 30.050 +/– 3.921, 38.211 +/– 1.721%, $F = 8.237$, $P = 0.000$). There was statistical difference in the MG63 cell apoptosis rate between the 4 groups in general, in other words, there was group effect ($F = 46.627$, $P = 0.000$). There was interaction between time factor and group factor ($F = 7.012$, $P = 0.000$). There was statistical difference in the number of migratory MG63 cells under the optical microscope (×200) between the 4 groups at 48 hours after the intervention (84.45 +/– 10.45, 74.56 +/– 9.45, 65.41 +/– 5.21, 40.21 +/– 4.52, $F = 148.243$, $P = 0.000$). The migratory MG63 cells was less in low – , middle – and high – concentration DCA – Na group compared to control group ($P = 0.017$, $P = 0.001$, $P = 0.000$), and the low – concentration DCA – Na group surpassed middle – and high – concentration DCA – Na group and middle – concentration DCA – Na group surpassed high – concentration DCA – Na group in the number of migratory MG63 cells ($P = 0.012$, $P = 0.001$, $P = 0.000$). **Conclusion**: DCA – Na can inhibit the proliferation of human MG63 osteosarcoma cells, increase the Caspase – 3 enzymatic activity of

MG63 cells,induce and accelerate the apoptosis of MG63 cells and inhibit the migration of MG63 cells. Moreover,the higher the concentration of DCA – Na is and the longer the intervention time is,the more obvious the effect is.

Key words osteosarcoma;dichloroacetate;cell proliferation;apoptosis;cell movement;caspase 3;MG63 cell lines

骨肉瘤是常见于青少年的一种恶性程度很高的原发性肿瘤[1-2]。该病尚无早期明确诊断的方法,且骨肉瘤细胞具有显著的迁移能力与局部迁移能力,因此,患者被确诊时多已发生骨远端转移或肺转移[3-4]。目前,骨肉瘤主要的治疗方法是手术和化学药物治疗。但传统的化疗药物不良反应较多,可给患者身心带来巨大伤害[5]。肿瘤细胞的能量供给主要依赖糖酵解,具有糖酵解过度活化的特性。二氯乙酸盐(dichloroacetate,DCA)可有效抑制线粒体丙酮酸脱氢酶激酶的活性,诱导细胞凋亡,抑制肿瘤细胞生长[6]。本实验观察二氯乙酸钠(sodium dichloroacetate,DCA – Na)对人骨肉瘤 MG63 细胞增殖、凋亡和迁移的影响,为 DCA – Na 用于骨肉瘤的治疗提供依据。

1 材料与仪器

人骨肉瘤 MG63 细胞株(郑州大学基础医学院提供),DCA – Na(Sigma – Aldrich 公司,美国),Cell Counting Kit – 8(CCK – 8)细胞活性检测试剂盒(东仁化学科技上海有限公司),Transwell 小室(Corning Costar 公司,美国),Annexin V – FITC 细胞凋亡检测试剂盒(上海碧云天生物技术有限公司),GIBCO® Roswell Park Memorial Institute – 1640(RPMI – 1640)培养基(赛默飞世尔科技中国公司)。二氧化碳恒温培养箱(Thermo Fisher 公司,美国),酶标仪(Bio – RAD 公司,美国),流式细胞仪(Eppendorf 公司,德国),台式离心机(Eppendorf 公司,德国),分光光度计(Thermo 公司,美国),–80 ℃超低温冰箱(青岛海尔股份有限公司),荧光显微镜(Olympus 公司,日本)。

2 方 法

2.1 细胞培养 复苏人骨肉瘤 MG63 细胞株,在 RPMI – 1640 培养基中加 10% 新生小牛血清(pH 值保持在 6.8 ~ 7.2),在 37 ℃、5% CO_2 培养箱中培养 48 h 后,用 0.25% 胰酶消化传代。

2.2 细胞增殖检测 选用对数生长期的 MG63 细胞接种于 24 孔培养板中,每孔加 RPMI – 1640 培养基 100 μL,细胞接种密度为 1×10^5 个 · mL^{-1},于 37 ℃、5% CO_2 培养箱中培养 24 h。将培养的细胞随机分为对照组和低、中、高浓度 DCA – Na 组,对照组不加 DCA – Na;低、中、高 DCA – Na 组加入 DCA – Na(终浓度分别为 50 μg · mL^{-1}、100 μg · mL^{-1}、200 μg · mL^{-1}),每组设 3 个重复孔,每块板设 1 个只加等量 RPMI – 1640 培养基不加细胞和 DCA – Na 的空白组。分别在干预 24 h、48 h、72 h 后,每孔加入 5 mg · mL^{-1} 的 CCK – 8 溶液 20 μL,摇床上摇动 1 min,在培养箱内继续孵育 2 h 后,用酶标仪在 450 nm 波长下测定光密度(optical density,OD),每组取平均值。计算低、中、高 DCA – Na 组细胞增殖抑制率,细胞增殖抑制率 = [1 –(DCA – Na 组 OD 值 – 空白组 OD 值)/(对照组 OD 值 – 空白组 OD 值)] × 100%。

2.3 细胞凋亡检测

2.3.1 Caspase – 3 活性检测 细胞接种、分组和加药方法同前。分别在干预 24 h、48 h、72 h 后按 Caspase – 3 活性检测试剂盒说明进行检测,空白组调零,分光光度计测定每组细胞 OD 值,即 Caspase 3 活性值,每组测 3 次,取平均值。

2.3.2 流式细胞法细胞凋亡检测 细胞接种、分组和加药方法同前。分别在干预 24 h、48 h、72 h 后,用胰酶消化后收集细胞,调整细胞密度至 1×10^6 个 · mL^{-1},用 200 μL 结合缓冲液重新悬浮细胞后,置于 5 mL 的流式管中,按 Annexin V – FITC 细胞凋亡检测试剂盒使用说明加入 2 μLAnnexinV – FITC 和 2 μLPI,混匀后避光孵育 15 min,1 h 内进行流式细胞仪检测,每组测 3 次,取平均值。

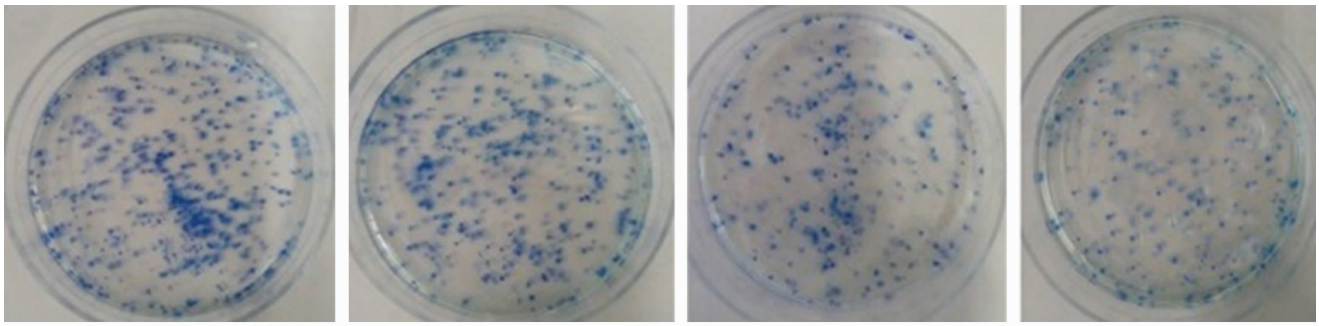
2.4 细胞迁移检测 将 Transwell 小室置于加入血清和 RPMI – 1640 培养基的 24 孔板中,取对数生长期 MG63 细胞,制作成浓度为 1.0×10^5 个 · mL^{-1} 的细胞悬液,将 200 μL 细胞悬液加入 Transwell 小室内,按前述方法分组、加药。37 ℃培养 48 h,取出小室去除培养基,多聚甲醛固定细胞后,用苏木精进行染色,显微镜下随机选择 3 个视野计数迁移细胞个数,取 3 个视野的平均值。

2.5 数据处理 应用 SPSS 18.0 统计软件处理数据。细胞增殖检测结果、Caspase – 3 酶活性检测结果和流式细胞法细胞凋亡检测结果的统计处理采用重复测量资料的方差分析;细胞迁移检测结果的组间总

体比较采用单因素方差分析,组间两两比较采用 LSD – t 检验。检验水准 $\alpha = 0.05$。

3　结　果

3.1　细胞增殖检测结果　干预后,低、中、高 DCA – Na 组 MG63 细胞增殖抑制明显(图 1)。低、中、高 DCA – Na 组 MG63 细胞增殖抑制率均呈上升趋势(图 2)。各时间点间的 MG63 细胞增殖抑制率差异有统计学意义($F = 847.080$,$P = 0.000$),存在时间效应;干预 24 h、48 h、72 h 后,各组间 MG63 细胞增殖抑制率的差异均有统计学意义,低 DCA – Na 组 < 中 DCA – Na 组 < 高 DCA – Na 组($F = 2.795$,$P = 0.000$;$F = 4.362$,$P = 0.000$;$F = 5.896$,$P = 0.000$);3 组间 MG63 细胞增殖抑制率总体比较,差异有统计学意义,存在分组效应($F = 296.412$,$P = 0.000$);时间因素与分组因素之间存在交互效应($F = 75.678$,$P = 0.000$);见表 1。



(1) 对照组　(2) 低DCA-Na组　(3) 中DCA-Na组　(4) 高DCA-Na组

图 1　干预 72 h 后不同浓度 DCK – Na 对人骨肉瘤 MG63 细胞增殖的影响情况(Giemsa 染色　×200)

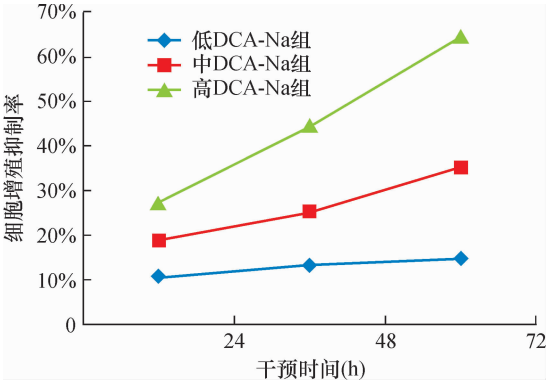


图 2　低、中、高 DCA – Na 组人骨肉瘤 MG63 细胞增殖抑制率变化趋势图

3.2　细胞凋亡检测结果

3.2.1　Caspase – 3 酶活性检测结果　对照组 MG63 细胞 Caspase – 3 酶活性无明显变化,干预后低、中、高浓度 DCA – Na 组 MG63 细胞 Caspase – 3 酶活性均呈上升趋势(图 3)。各时间点间 MG63 细胞 Caspase – 3 酶活性 OD 值的差异有统计学意义($F = 1\,480.792$,$P = 0.000$),存在时间效应;干预 24 h、48 h、72 h 后,各组间 MG63 细胞 Caspase – 3 酶活性的差异均有统计学意义,且对照组 < 低 DCA – Na 组 < 中 DCA – Na 组 < 高 DCA – Na 组($F = 2.320$,$P = 0.000$;$F = 1.014$,$P = 0.000$;$F = 1.000$,$P = 0.000$);4 组间 MG63 细胞 Caspase – 3 酶活性总体比较,差异有统计学意义,存在分组效应($F = 624.975$,$P = 0.000$);时间因素与分组因素之间存在交互效应($F = 94.579$,$P = 0.000$);见表 2。

表 1　低、中、高 DCA – Na 组人骨肉瘤 MG63 细胞增殖抑制率比较

组别	干预后各时间点 MG63 细胞增殖抑制率($\bar{x} \pm s$)				F 值	P 值
	24 h	48 h	72 h	合计		
低 DCA – Na 组	(10.802 ± 3.000)%	(13.098 ± 1.299)%	(14.728 ± 1.177)%	(12.876 ± 4.287)%	6.392	0.033
中 DCA – Na 组	(18.792 ± 2.261)%	(25.215 ± 2.676)%	(35.297 ± 4.757)%	(26.435 ± 3.231)%	64.255	0.000
高 DCA – Na 组	(27.080 ± 3.133)%	(44.382 ± 2.397)%	(64.227 ± 4.549)%	(45.230 ± 3.359)%	45.079	0.000
合计	(18.892 ± 2.798)%	(27.565 ± 2.124)%	(38.084 ± 3.494)%	(28.165 ± 2.805)%	847.080[1)]	0.000[1)]
F 值	2.795	4.362	5.896	296.412[1)]	($F = 75.678$, $P = 0.000$)[2)]	
P 值	0.000	0.000	0.000	0.000[1)]		

1)主效应的 F 值和 P 值;2)交互效应的 F 值和 P 值

3.2.2　流式细胞法细胞凋亡检测结果　对照组 MG63 细胞未见明显凋亡,干预后低、中、高 DCA – Na 组 MG63 细胞凋亡明显(图 4),对照组细胞凋亡率变化不明显,低、中、高 DCA – Na 组细胞凋亡率均呈上

升趋势（图 5）。各时间点间 MG63 细胞凋亡率的差异有统计学意义（$F=359.645$，$P=0.000$），存在时间效应；干预 24 h、48 h、72 h 后，各组间 MG63 细胞凋亡率的差异均有统计学意义，且对照组 < 低 DCA - Na 组 < 中 DCA - Na 组 < 高 DCA - Na 组（$F=6.733$，$P=0.000$；$F=9.292$，$P=0.000$；$F=8.237$，$P=0.000$）；4 组间 MG63 细胞凋亡率总体比较，差异有统计学意义，存在分组效应（$F=46.627$，$P=0.000$）；时间因素与分组因素之间存在交互效应（$F=7.012$，$P=0.000$）。见表 3。

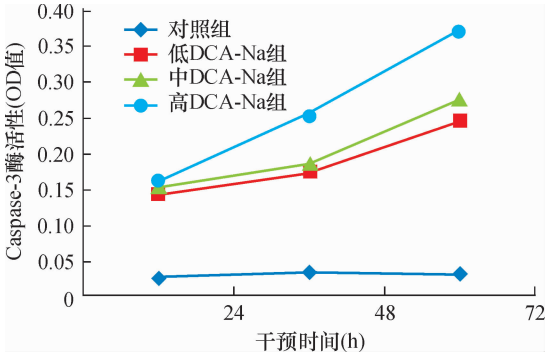
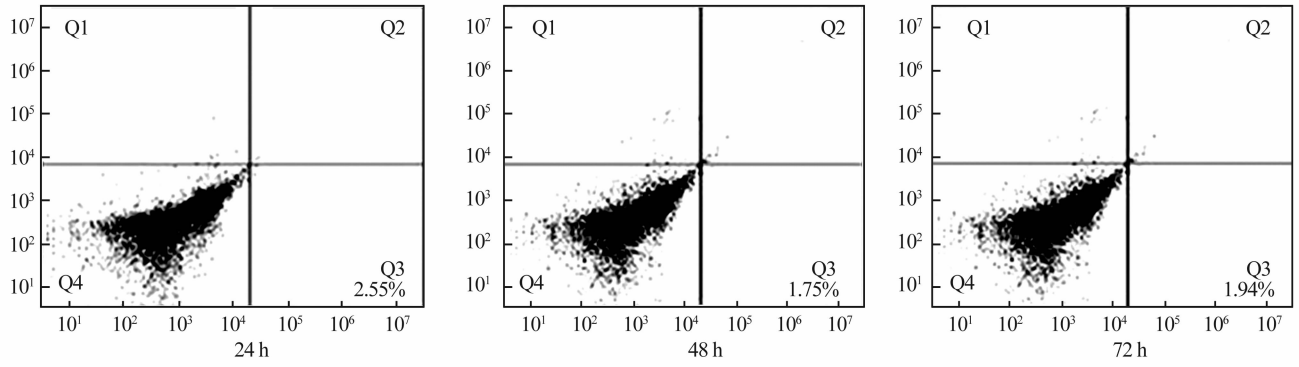


图 3　4 组人骨肉瘤 MG63 细胞 Caspase - 3 酶活性变化趋势图

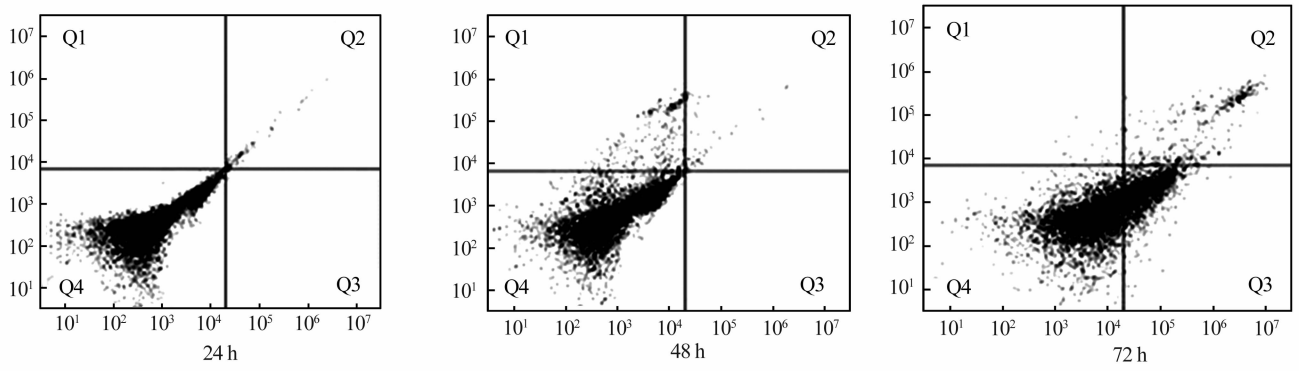
表 2　4 组人骨肉瘤 MG63 细胞 Caspase - 3 酶活性比较

组别	干预后各时间点 MG63 细胞 Caspase - 3 酶活性 OD 值（$\bar{x}\pm s$）				F 值	P 值
	24 h	48 h	72 h	合计		
对照组	0.027 ± 0.003	0.035 ± 0.003	0.031 ± 0.004	0.031 ± 0.015	0.743	0.514
低 DCA - Na 组	0.143 ± 0.005	0.174 ± 0.004	0.246 ± 0.006	0.188 ± 0.002	112.054	0.000
中 DCA - Na 组	0.153 ± 0.008	0.184 ± 0.007	0.275 ± 0.003	0.204 ± 0.003	461.562	0.000
高 DCA - Na 组	0.161 ± 0.003	0.253 ± 0.001	0.371 ± 0.004	0.261 ± 0.008	375.681	0.000
合计	0.121 ± 0.003	0.161 ± 0.006	0.231 ± 0.017	0.171 ± 0.007	1 480.792[1)]	0.000[1)]
F 值	2.320	1.014	1.000	624.975[1)]	（$F=94.579$，	
P 值	0.000	0.000	0.000	0.000[1)]	$P=0.000$）[2)]	

1）主效应的 F 值和 P 值；2）交互效应的 F 值和 P 值



(1) 对照组



(2) 低DCA-Na组

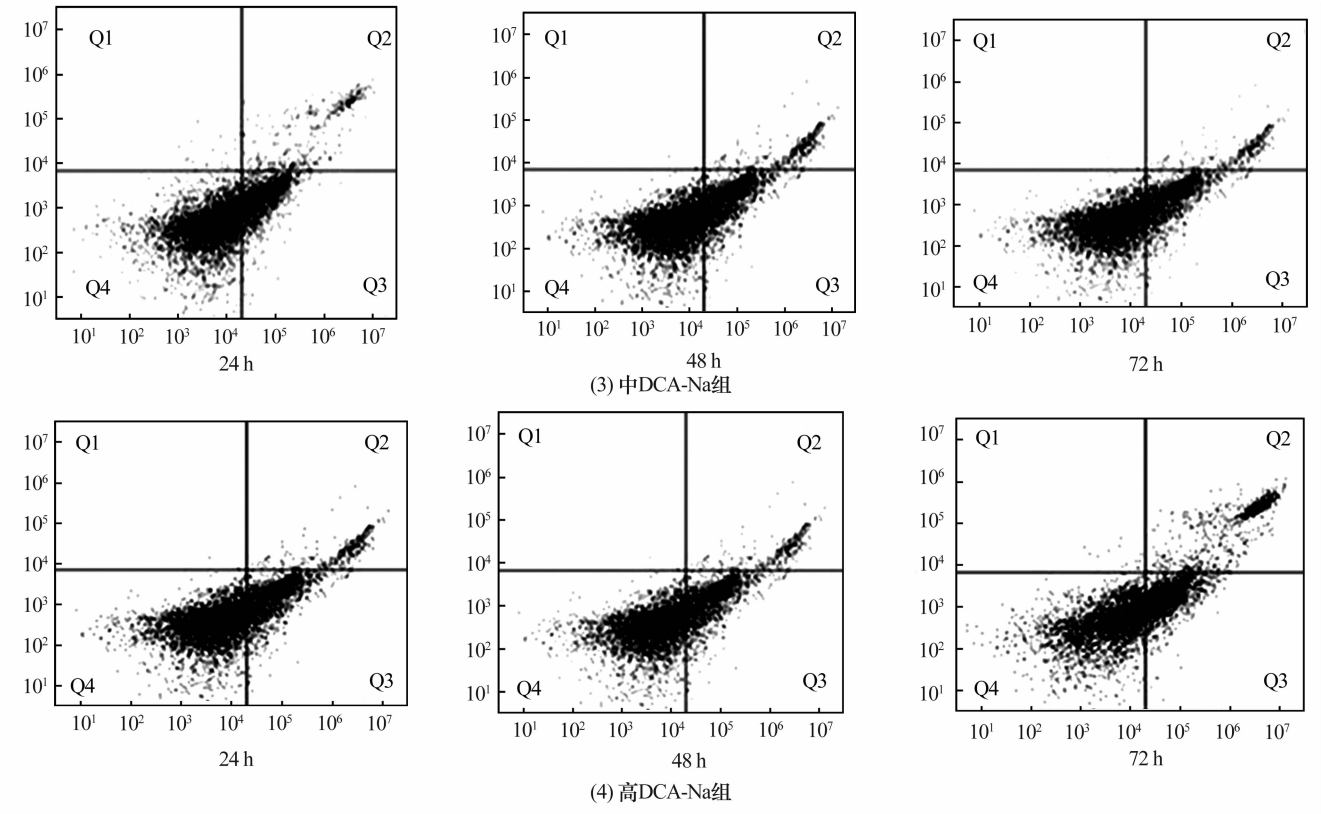


图 4　4 组骨肉瘤 MG63 细胞流式细胞法细胞凋亡检测情况

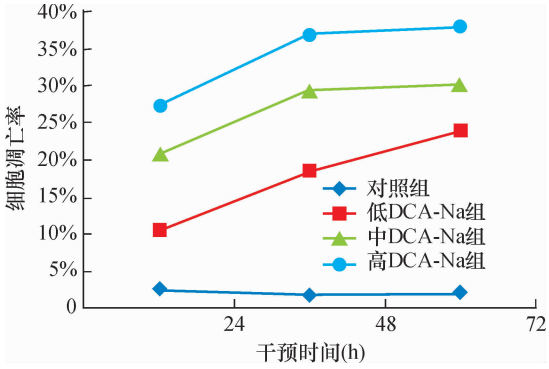


图 5　4 组人骨肉瘤 MG63 细胞凋亡率变化趋势图

3.3　细胞迁移检测结果　干预 48 h 后,4 组迁移细胞数[显微镜每个视野下(×200)]的组间差异有统计学意义[(84.45±10.45)个,(74.56±9.45)个,(65.41±5.21)个,(40.21±4.52)个,F=148.243,P=0.000)];低、中、高 DCA-Na 组迁移细胞数均少于对照组(P=0.017,P=0.001,P=0.000),且低 DCA-Na 组>中 DCA-Na 组>高 DCA-Na 组(P=0.012,P=0.001,P=0.000)。见图 6。

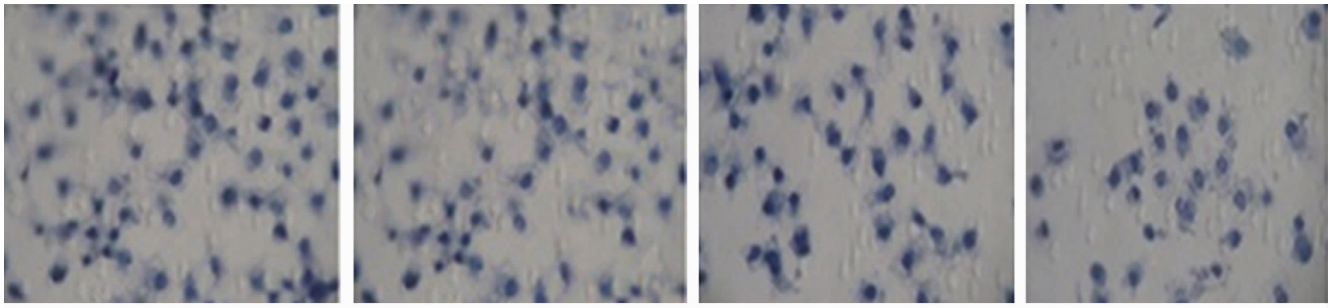
表 3　4 组人骨肉瘤 MG63 细胞凋亡率比较

组别	干预后各时间点细胞凋亡率($\bar{x}\pm s$)				F 值	P 值
	24 h	48 h	72 h	合计		
对照组	(2.554±0.427)%	(1.748±0.202)%	(1.944±0.112)%	(2.082±0.317)%	8.251	0.102
低 DCA-Na 组	(10.708±2.012)%	(18.604±2.721)%	(24.071±3.921)%	(17.794±2.885)%	17.295	0.003
中 DCA-Na 组	(20.857±2.531)%	(29.471±1.605)%	(30.050±3.921)%	(26.870±2.696)%	40.954	0.024
高 DCA-Na 组	(27.312±2.140)%	(36.873±2.734)%	(38.211±1.721)%	(34.133±2.198)%	32.297	0.029
合计	(15.918±1.776)%	(21.337±1.816)%	(23.560±2.419)%	(21.271±1.000)%	359.645[1)]	0.000[1)]
F 值	6.733	9.292	8.237	46.627[1)]	(F=7.012,	
P 值	0.000	0.000	0.000	0.000[1)]	P=0.000)[2)]	

1)主效应的 F 值和 P 值;2)交互效应的 F 值和 P 值

4　讨　论

无论是在有氧条件还是无氧条件下,肿瘤细胞对糖酵解的依赖与其恶性程度有关[7],骨肉瘤细胞的一大特性就是其主要供能方式是糖酵解[8-9]。活跃的



(1) 对照组　(2) 低DCA-Na组　(3) 中DCA-Na组　(4) 高DCA-Na组

图 6　4 组人骨肉瘤 MG63 细胞 Transwell 迁移实验结果（苏木精染色　×200）

糖酵解代谢是恶性肿瘤细胞显著的生化特征，是肿瘤细胞能量的重要来源，而探索通过干预糖酵解代谢途径来治疗恶性肿瘤的策略正越来越受瞩目[10]。

DCA－Na 是一种糖酵解抑制剂，临床常用于糖尿病乳酸中毒。DCA－Na 能够抑制线粒体内丙酮酸激酶的活性，降低糖酵解速率，同时使线粒体去极化，升高活性氧浓度、促进线粒体内凋亡诱导因子的外流，促进肿瘤细胞凋亡[11]。研究[12－15]表明 DCA－Na 在体外可抑制人肺腺癌 A549 细胞、人乳腺癌 MCF－7 细胞、恶性胶质瘤 M059K 细胞活性，促进细胞凋亡。本研究结果表明，DCA－Na 可抑制人骨肉瘤 MG63 细胞的增殖，增强 MG63 细胞 Caspase－3 酶活性，诱导和促进 MG63 细胞凋亡，抑制 MG63 细胞的迁移；且浓度越高、干预时间越长，其影响越明显。

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