JOURNAL OF OTOLARYNGOLOGY: RESEARCH Open Access

SCIENTIFIC LITERATURE

RESEARCH ARTICLE

Gadolinium Chloride (GdCl3) Inhibits Cell Proliferation and Cell Motilityin Cisplatin-Resistant Human Oral Cancer CAR Cells: A Novel Screening System for Real Time Imaging Using incuCyte™ Kinetic Live Cell Imaging System

Yuh-Feng Tsai[1,2#], Chi-Cheng Lu[3#], Fuu-Jen Tsai[4,5,6], Shih-Chang Tsai[7], Jo-Hua Chiang[8], Yu-Jen Chiu[9], Yan-Ru Peng[10] and Jai-Sing Yang[11**]

[1]Department of Diagnostic Radiology, Shin-Kong Wu Ho-Su Memorial Hospital, Taiwan
[2]School of Medicine, Fu-Jen Catholic University, Taiwan
[3]Department of Pharmacy, Buddhist Tzu Chi General Hospital, Taiwan
[4]Genetics Center, Department of Medical Research, China Medical University Hospital, Taiwan
[5]School of Chinese Medicine, China Medical University, Taiwan
[6]Department of Medical Genetics, China Medical University Hospital, Taiwan
[7]Department of Biological Science and Technology, China Medical University, Taiwan
[8]Department of Nursing, Chung-Jen Junior College of Nursing, Health Sciences and Management, Taiwan
[9]Division of Reconstructive and Plastic Surgery, Department of Surgery, Taipei Veterans General Hospital, Taiwan
[10]Schoolof Medicine, China Medical University, Taiwan
[11]Department of Medical Research, China Medical University Hospital, China Medical University, Taiwan
#equally contributed to both authors

ARTICLE INFO

Article history:
Received: 15 May 2017
Accepted: 28 July 2017
Published: 04 August 2017

Keywords:
Gadolinium chloride (GdCl3);
Cisplatin-resistant human oral cancer CAR cells;
Cell proliferation;
Cell motility;
IncuCyte kinetic live cell imaging system

Copyright: © 2017 Tsai YF et al., J Otolaryngol Res
This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Citation this article: Tsai YF, Lu CC, Tsai FJ, Tsai SC, Chiang JH et al,. Gadolinium Chloride (GdCl3) Inhibits Cell Proliferation and Cell Motilityin Cisplatin-Resistant Human Oral Cancer CAR Cells: A Novel Screening System for Real Time Imaging Using incuCyte™ Kinetic Live Cell Imaging System. J Otolaryngol Res. 2017; 1(1):112.

Correspondence:
Jai-Sing Yang,
Department of Medical Research, China Medical University Hospital, China Medical University, No. 2, Yuh-Der Road, Taichung City 40447, Taiwan,
Email: jaisingyang@gmail.com

ABSTRACT

Gadolinium chloride (GdCl3) is a contrast medium for Magnetic Resonance Imaging (MRI) use. Previously, our study has demonstrated that GdCl3 significantly reduced cell viability and triggered apoptosis in human osteosarcoma cellsby death receptor, mitochondria-dependent and Endoplasmic Reticulum (ER) stress pathways. However, no report showed theanticancer effects of GdCl3on drug-resistant oral cancer cells. In the present study, we investigated the anti-proliferation and anti-motility effects of GdCl3on thecisplatin-resistant human oral cancer CAR cells. Our results showed that GdCl3 inhibited cell proliferation and cell motility in a time-dependent manner by using IncuCyte™ Kinetic Live Cell Imaging System. Our finding provides direct evidence of real-time imaging analysis to support the oral anti-cancer effects of GdCl3on drug-resistant oral cancer cells in vitro.

Introduction

Oral cancer is the fifth leading cause of death in cancer from the annual report of the Ministry of Health and Welfare, R.O.C. (Taiwan) in 2014 [1]. Chemotherapy, radiotherapy, and surgery are major treatments for oral cancer [1-4]. However, drug resistance to the chemotherapeutic agentsis a major impediment in clinical medical treatment [4,5]. It is urgent for the discovery of a new compound to overcomeresistance of chemotherapeutic drugs in oral cancer. Gadolinium (Gd), a member of Lanthanides familyelement, has multi-biological effects and applications in Magnetic Resonance Imaging (MRI) contrast medium or the potential anti-cancer agents [6,7]. Gadolinium chloride (GdCl3) has demonstrated to inhibit cell proliferation and to induce mitochondria-dependent apoptosis in human hepatoblastoma HepG2 cells [7]. Our previous studyhas shown that GdCl3

triggered U-2 OS cell apoptosis through death receptor, mitochondria and ER stress-dependent pathways [6]. This study is to investigatethe anti-cancer effects of GdCl3on cell proliferation and cell motilityofcisplatin-resistant human oral cancer CAR cells.

Materials and Methods

1. Chemicals and reagents

Gadolinium chloride (GdCl3) was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), L-glutamine, and penicillin/streptomycin were obtained from Thermo Fisher Scientific (Waltham, MA, USA).

2. Cell culture

The cisplatin-resistant cells (CAR) was developed by treating CAL 27 cell line, a parental human tongue squamous cell carcinoma (American Type Culture Collection, Manassas, VA, USA) with 10 to 80 μM of cisplatin. The cells were cultured in DMEM fortified with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamineat 37°C wit h a humidified 5% CO2 air. The cisplatin-resistant CAR cells were constantly cultured in complement medium containing 80 μM cisplatin, unless otherwise indicated [1,2,4,5].

3. Cell proliferation assay by IncuCyte™ kinetic live cell imaging system

To measure the cell proliferation, CAR cells (2×104 cells) were plated into a 96-well plate and then incubated with or without 100 μM of GdCl3. Cell proliferationwas determined by the IncuCyte™ Kinetic Live Cell Imaging System (Essen BioScience, Ann Arbor, MI, USA). Imaging was photographed every 2 h for a 48-h period [1,8].

4. Cellmotility assay by IncuCyte™ Kinetic Live Cell Imaging System

Cell motility assaywas analyzed using wound healing assay and by IncuCyte™ Kinetic Live Cell Imaging System. CAR cells wereplated in 96-well plates (~1×105cells/well) and incubated to 90% confluence. After scratch wounds were made using the Incucyte Scratch instrument (Essen Bio Science), cells were treated with or without 50 μM of GdCl3. Cell motility was determined by the IncuCyte™ Kinetic Live Cell Imaging System (Essen Bio Science). Imaging was photographed every 3 h for a 48-h period [4,9].

Results and Discussion

After CAR cells were treated with GdCl3 at100 μM, cell proliferation was assessed by IncuCyte™ Kinetic Live Cell Imaging System. Cell proliferation wasdecreased in GdCl3-treated CAR cells in a concentration-dependent manner (Figure 1 and Supplementary video 1).

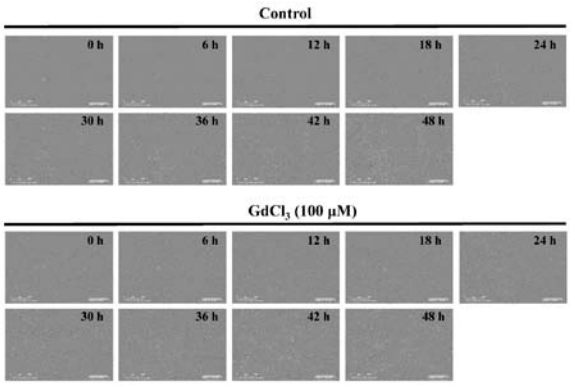
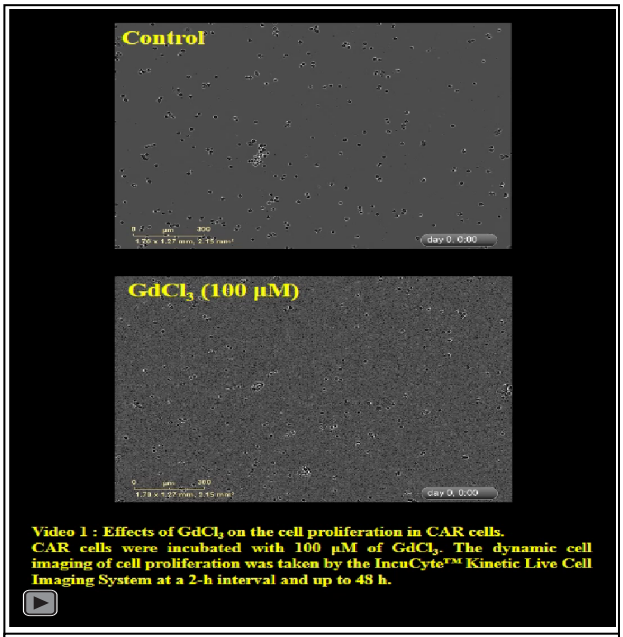


Figure 1: Effects of gadolinium chloride ($GdCl_3$) on cell proliferation in cisplatin-resistant human oral cancer CAR cells. Cells were treated with or without 100 μM GdCl3for 24 h. Cell proliferationimaging was determined by the IncuCyte™ Kinetic Live Cell Imaging System.Imaging was photographedevery 6 hfor a 48-h period.



Supplementary Video 1: Effects of gadolinium chloride ($GdCl_3$) on cell proliferation in cisplatin-resistant human oral cancer CAR cells.Cells were incubated with or without100 μM of $GdCl_3$. Cell proliferationimaging was determined by the IncuCyte™ Kinetic Live Cell Imaging System.Imaging was photographed every 2 h for a 48-h period.

When cells were treated withGdCl3 at 100 μM, the morphological changes, detachment from the surface,

and some cell debriswere observed in CAR cells.Our data suggested that GdCl3 inhibited cell proliferation in CAR cells.In addition, CAR cells were exposed to 50 μMof GdCl3, cell motility was reduced in CAR cells, and this effect was in a time-dependent manner (Figure 2 and Supplementary video 2).

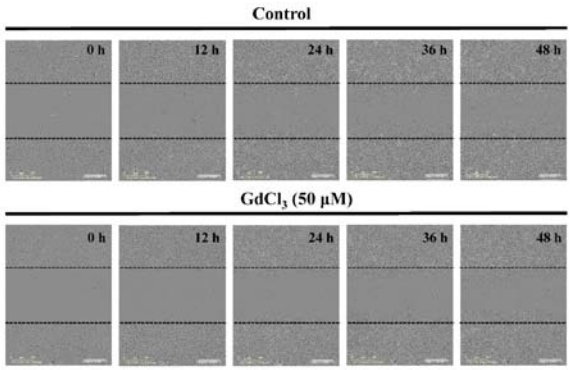
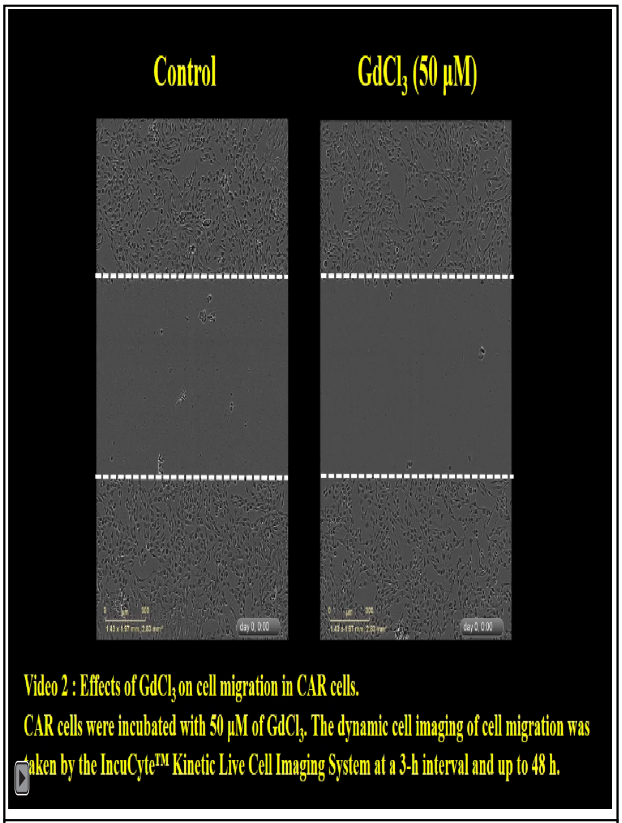


Figure 2: Effects of gadolinium chloride ($GdCl_3$) on cell motility in cisplatin-resistant human oral cancer CAR cells. Cells were treated with or without 50 μM$GdCl_3$for 24 h. Cell motilityimaging was determined by the IncuCyte™ Kinetic Live Cell Imaging System.Imaging was photographed every 12 h for a 48-h period.



Supplementary Video 2: Effects of gadolinium chloride ($GdCl_3$) on cell motility in cisplatin-resistant human oral cancer CAR cells. Cell motilityimaging was determined by the IncuCyte™ Kinetic Live Cell Imaging System.Imaging was photographed every 3 h for a 48-h period.

Our results indicated that high concentration of GdCl3 caused growth inhibition and low concentration of GdCl3 caused antimotility in CAR cells. Lanthanides (Lns) compounds haveanti-cancer activities such as anti-proliferation, promotion of cell cycle progression, antimotility and induction of cell apoptosis [6,10-13]. This study is first to report that GdCl3 can be successfully functional to inhibit cell proliferation and motilityin cisplatin-resistant human oral cancer CAR cells.

It is important and essential for anti-cancer drug discoveryto measure the cell proliferation rate in an in vitro study [14-17]. Many strategies and methods have been proposed to detect cell proliferation rates. MTT and WST-1 have been widely used to detect the overall metabolic activity in cells [18-20]. However, those methods cannot provide directevidenceof real-time imaging analysis [1]. In the current study, we are the first time to use the IncuCyte™ Kinetic Live Cell Imaging System for characterizing cell proliferation and cell motility in GdCl3-treated CAR cells. In the future, the anti-proliferation and antimotility activities of GdCl3 willbe further studied by using this powerful tool IncuCyte™ Kinetic Live Cell Imaging System in our group.

Conclusion

In conclusion, we herein demonstrated that GdCl3 exhibits direct anti-cancer activity by suppression of cell proliferation and cell motilityin cisplatin-resistant human oral cancer CAR cells.

Acknowledgment

This work was supported by the grant from China Medical University Hospital, Taichung, Taiwan (DMR-106-122). The authors also would like to thank Mr. Meng-Jou Liao and Mr. Chin-Chen Lin (Tekon Scientific Corp., Taipei, Taiwan) for the excellent technique supports and providing partial instruments.

References

1. Lee MR, Lin C, Lu CC, Kuo SC, Tsao JW, et al. (2017). YC-1 induces G0/G1 phase arrest and mitochondria dependent apoptosis in cisplatin-resistant human oral cancer CAR cells. Biomedicine (Taipei). 7: 31-42.

2. Chang CH, Lee CY, Lu CC, Tsai FJ, Hsu YM, et al. (2017). Resveratrol-induced autophagy and apoptosis in cisplatin-resistant human oral cancer CAR cells: A key role of AMPK and Akt/mTOR signaling. Int J Oncol. 50: 873-882.

3. Yuan CH, Horng CT, Lee CF, et al. (2017). Epigallocatechin gallate sensitizes cisplatin-resistant oral cancer CAR cell apoptosis and autophagy through stimulating AKT/STAT3 pathway and suppressing multidrug resistance 1 signaling. Environ Toxicol. 32: 845-855.

4. Chang PY, Peng SF, Lee CY, Lu CC, Tsai SC, et al. (2013). Curcumin-loaded nanoparticles induce apoptotic cell death through regulation of the function of MDR1 and reactive oxygen species in cisplatin-resistant CAR human oral cancer cells. Int J Oncol. 43: 1141-1150.

5. Hsieh MT, Chen HP, Lu CC, Chiang JH, Wu TS, et al. (2014). The novel pterostilbene derivative ANK-199 induces autophagic cell death through regulating PI3 kinase class III/beclin 1/Atgrelated proteins in cisplatinresistant CAR human oral cancer cells. Int J Oncol. 45: 782-794.

6. Tsai YF, Huang CW, Chiang JH, et al. (2016). Gadolinium chloride elicits apoptosis in human osteosarcoma U-2 OS cells through extrinsic signaling, intrinsic pathway and endoplasmic reticulum stress. Oncol Rep. 36: 3421-3426.

7. Pagliara P, Carla EC, Caforio S, Chionna A, Massa S, et al. (2003). Kupffer cells promote lead nitrate-induced hepatocyte apoptosis via oxidative stress. Comp Hepatol. 2: 8.

8. Kerz M, Folarin A, Meleckyte R, Watt FM, Dobson RJ. (2016). A Novel Automated High-Content Analysis Workflow Capturing Cell Population Dynamics from Induced Pluripotent Stem Cell Live Imaging Data. J Biomol Screen. 21: 887-896.

9. Drebert Z, MacAskill M, Doughty-Shenton D, Karolien De Bosscher, Marc Brack, et al. (2017). Colon cancer-derived myofibroblasts increase endothelial cell migration by glucocorticoid-sensitive secretion of a pro-migratory factor. Vascul Pharmacol. 89: 19-30.

10. Zhu F, Li X, Jiang Y, et al. (2015). GdCl3 suppresses the malignant potential of hepatocellular carcinoma by inhibiting the expression of CD206 in tumorassociated macrophages. Oncol Rep. 34: 2643-2655.

11. Wang P, Zou XM, Huang J, Zhang TL, Wang K. (2011). Gadolinium inhibits prostate cancer PC3 cell migration and suppresses osteoclast differentiation in vitro. Cell Biol Int. 35: 1159-1167.

12. Lazar G, Farkas G, Csanadi J, Lazar G. (1997). Gadolinium chloride-induced macrophage blockade prevents rejection of human insulinoma cell xenograft in rats. Transplantation. 63: 729-732.

13. Bear CE, Li CH. (1991). Calcium-permeable channels in rat hepatoma cells are activated by extracellular nucleotides. Am J Physiol. 261: C1018-1024.

14. Marin de Mas I, Aguilar E, Jayaraman A, et al. (2014). Cancer cell metabolism as new targets for novel designed therapies. Future Med Chem 6: 1791-1810.

15. Taylor P, Colman L, Bajoon J. (2014). The search for plants with anticancer activity: pitfalls at the early stages. J Ethnopharmacol. 158: 246-254.

16. Pandey S, Shaw PN, Hewavitharana AK. (2015). Review of procedures used for the extraction of anti-cancer compounds from tropical plants. Anticancer Agents Med Chem. 15: 314-326.

17. Cooper EL, Yao D. (2012). Diving for drugs: tunicate anticancer compounds. Drug Discov Today. 17: 636-648.

18. Bahadar H, Maqbool F, Niaz K, Abdollahi M. (2016). Toxicity of Nanoparticles and an Overview of Current Experimental Models. Iran Biomed J. 20: 1-11.

19. Tournebize J, Sapin-Minet A, Bartosz G, Leroy P, Boudier A. (2013). Pitfalls of assays devoted to evaluation of oxidative stress induced by inorganic nanoparticles. Talanta. 116: 753-763.

20. Berridge MV, Herst PM, Tan AS. (2005). Tetrazolium dyes as tools in cell biology: new insights into their cellular reduction. Biotechnol Annu Rev. 11: 127-152.