Cancer Cell Research 12 (2016) 294-298

Available at http:// www.cancercellresearch.org ISSN 2161-2609

The Influences of Chemotherapeutics on the Cell Cycle in SK-N-SH Neuroblastoma Cells and Clinic Significance

Xilin Xiong[1,*], Ling Liu[2], Kai Qi[1], Chi Zhang[1], Yueqin Chen[3], Yang Li[1]

[1]Pediatric Hematology/Oncology, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, 510120, China

[2]Department of Pediatric Hematology/Oncology, Affiliated Hospital of Guangdong Medical College, Zhanjiang, 524000, China

[3]Department of Life Sciences of Sun Yat-Sen University, Guangzhou 510120, China

Abstract: **This study was aimed to confirm the drug influence on cell cycle of SK-N-SH cells by flow cytometry analysis. The SK-N-SH cells were divided into two groups including Treatment group and control group. All of them were given chemotherapy drugs of ADM, CTX, VP16 and VCR, and then detected the influences of chemotherapy drugs on the cell cycle of SK-N-SH cells by flow cytometry instrument and. ADM: The percentage of G0/G1 phase of experimental group is (51.41 ±0.13)%, the percentage of G0/G1 phase of control group is (16.59±0.52)%. CTX：The percentage of G0/G1 phase of experimental group is (35.19 ±0.69)%, the percentage of G0/G1 phase of control group is (9.03±0.21)%. VP16： The percentage of G0/G1 phase of experimental group is (50.25 ±2.06)%, the percentage of G0/G1 phase of control group is (19.33±1.12)%. DDP： The percentage of G0/G1 phase of experimental group is (25.24 ±0.37)%, the percentage of G0/G1 phase of control group is (13.51±0.39)%. After treated with ADM, CTX, VP16 and DDP, the cell number of G0/G1 phase is more than control group in SK-N-SH cells, this showed significant difference (P<0.01). VCR： The percentage of G2/M phase of experimental group is (45.76±0.39)%, the percentage of G2/M phase of control group is (10.95±0.36)%. The cell number of G2/M phase is more than control group in SK-N-SH cells, this showed significant difference (P<0.01). ADM, CTX, VP16 and DDP can significantly arrest cell in G0/G1 phase, while VCR can significantly arrest cell in G2/M phase.**

Keywords: Neuroblastoma; Cell Cycle; Chemotherapeutics

Received 29 September 2016, Revised 23 October 2016, Accepted 25 October 2016

*Corresponding Author: Xilin Xiong, kikibear@163.com

1. Introduction

Neuroblastoma (neuroblastoma, NB) is one of the most common extracranial solid tumors of childhood, accounted for 8%~10% in malignant tumor of child [1]. Its originated from neural crest cells, and can occur in any part of the sympathetic nervous system. Neuroblastoma has high malignant degree and bad progress [2].

NB treatment is mainly depended on multi-disciplinary comprehensive treatment, including surgery, chemotherapy, radiotherapy and immunotherapy. Stem cell transplantation, gene therapy, etc, but the most effective means of NB is still the chemotherapy. The effective chemotherapy scheme for NB recognized at domestic and overseasat at present stage was CAV+PVP, and the main drug composition were cyclophosphamide (CTX), adriamycin (ADM), vincristine (VCR), cisplatin (DDP) and etoposide/teniposide (VP16/ VM26) [3-6]. CTX is a bifunctional alkylating agent and cell cycle non-specific drug, it happens with the DNA cross-links, and can inhibit DNA synthesis. While ADM effects on DNA and inhibits the synthesis of nucleic acids [8]. VCR mainly inhibit tubulin polymerization and to influence the formation of spindle microtubules, so as to the mitotic stop in the middle. DDP mainly acts on the purine and pyrimidine of DNA which can inhibit the synthesis of nucleic acids [9]. VP16 main acting on the DNA topoisomerase Ⅱ, forming stability reversible complexes of drug-enzyme-DNA, and prevent DNA repair [6]. Through positive and effective chemotherapy, many early NB children receiving incomplete surgical removal can obtain secondary surgery opportunity. The chemotherapy resection rate can reach to 74.1%, but almost 30% children was still poor first-line chemotherapy curative effect. It may be related to resistance by stimulating resistance gene expression or other related gene expression affected the prognosis [10].

In this study, by detection of ADM, CTX, VCR, we discussed the change rule and clinical significance of VP16 and DDP NB SK-N-SH in the role of the cell cycle.

2. Materials and Methods

2.1. The experiment reagent and instrument

Adriamycin (ADM), D1515, 10mg, Sigma Company. Cyclophosphamide (CTX), SU2081, 1g, the Sigma Company. Vincristine (VCR), 94898, 1mg, the Sigma Company. Etoposide (VP16), E1383, 25mg, Sigma Company. Cisplatin (DDP), P4394, 25mg, Sigma Company. Anti-TrkA antibody (μl/100), Anti-TrkB

antibody (μl/100), Anti-Trk C antibody (μl/100) were Abcam products. Anti-rabbit IgG antibody (μl/100), Anti-mouse IgG antibody (μl/100) products are Sigma company. High-speed refrigerated centrifuge Promega Company (type of J-25). Flow cytometry (FCM-2012) the BD Company in America.

2.2. Cell sources

SK-N-SH cells: human neuroblastoma cell lines, purchased from cell bank of Zhongshan School of Medicine.

2.2.1. Cell culture

Join the 10% of inactivated fetal bovine serum in the DMEM/F12 1:1 mix medium, and placed in saturated humidity 5% CO_2 incubator to culture and batches for 3-4 days.

2.2.2. Detect the cell cycle with flow cytometry instrument

Treatment group: add cells and different concentration of the drug. Control group: only add complete medium and cells. Logarithmic phase SK-N-SH cells were taken and vaccinated with 3×105/ml cell concentration in 6 orifice, cultivated for 24~48h. Treatment group were drug with concentration which according to the results of the CCK8 experimental, while control group added volume DMEM/F12 culture only. Cells were cultured in 37℃ 5% CO_2 incubator for 24h and 48h. Digestion with 0.125% of trypsin, washing twice with 4℃ precooling PBS, and then 70% fixated with cold ethanol. Cells were added binding-buffer 200μl, plus Annexin V-10μl, PI 5μl, avoid light effected after 15min, added the binding-300μl buffer. Detect cell cycle phase distribution with flow cytometry instrument within 1hour.

Table. 1 The change of SK-N-SH/% cells after using ADM, CTX, VP16 and DDP.

Cell cycle	ADM		CTX		VP16		DDP	
	NC	2.5μg/ml	NC	2.5μg/ml	NC	2.5μg/ml	NC	2.5μg/ml
G0/G1	16.59±0.5	51.41±0.13*	9.03±0.21	35.19±0.69*	19.33±1.12	50.25±2.06*	13.51±0.39	25.24±0.37*
S	73.23±0.9	30.13±0.42	77.05±0.38	41.65±0.25	55.62±4.67	1.38±0.17	73.73±0.24	56.64±0.44
G2/M	9.65±0.40	17.92±0.35	5.90±0.28	18.33±0.58	16.84±2.40	18.03±0.67	10.95±0.36	16.18±0.14

*Change of G0/G1 phase between different concentrations group, P＜0.01

2.3. Statistical methods

Data processed by SPSS17.0 statistical software, the comparison of multiple sets of independent samples using single factor analysis of variance or nonparametric test, P<0.05 for the significant difference, P<0.01 for the extremely significant difference.

3. Results

3.1. The influence of ADM, CTX, VP16 and DDP to SK-N-SH on the cell cycle

By literature and early damage toxicity test results, we disposed SK-N-SH cell for 48 hours with 2.5μg/ml ADM, 3mg/ml CTX, 100μg/ml VP16 and 5μg/ml DDP. Flow cytometry instrument testing shows that: (1) ADM: treatment group G0/G1 phase percentage is (51.41+0.13)%, control group in G0/G1 phase percentage (16.59+0.52)%. (2) the CTX: treatment group G0/G1 phase percentage (35.19+0.69)%, control group in G0/G1 phase percentage (9.03+0.21)%. (3) VP16: treatment group G0/G1 phase Percentage (50.25+2.06)%, control group in G0/G1 phase percentage (19.33+1.12)%. (4) DDP: treatment group G0/G1 phase percentage is (25.24+0.37)%, control group in G0/G1 phase percentage (13.51+0.39)%. After dialed with ADM, CTX, VP16 and DDP, compared with the control group (NC), G0/G1 phase cell number increased in treatment group, and it have significant difference in the two groups (P<0.01). It shows that the cell cycle can be obviously block in G0/G1 phase. (Table 1, Figure 1).

3.2. The influence of VCR to SK-N-SH cell cycle

By literature and early damage toxicity test results, 150ng/ml VCR were used on SK-N-SH cell for 72h, flow cytometry instrument testing shows, VCR: treatment group G2/M phase percentage for (45.76+0.39)%, control group G2/M phase percentage for (10.95+0.36)%. Compared with the control group (NC), G2/M phase cells was significant increased (P<0.01). It shows that the cell cycle can be obviously block in G2/M phase (Table2, Figure 2).

Table 2. The cell cycle change of SK-N-SH/% cells after using VCR.

VCR	G0/G1	S	G2/M**
NC	73.73±0.24	13.51±0.39	10.95±0.36
150ng/μl-72h	14.54±0.71	21.93±0.23	45.76±0.39

** Change of G0/G1 phase between different concentrations group, P＜0.01.

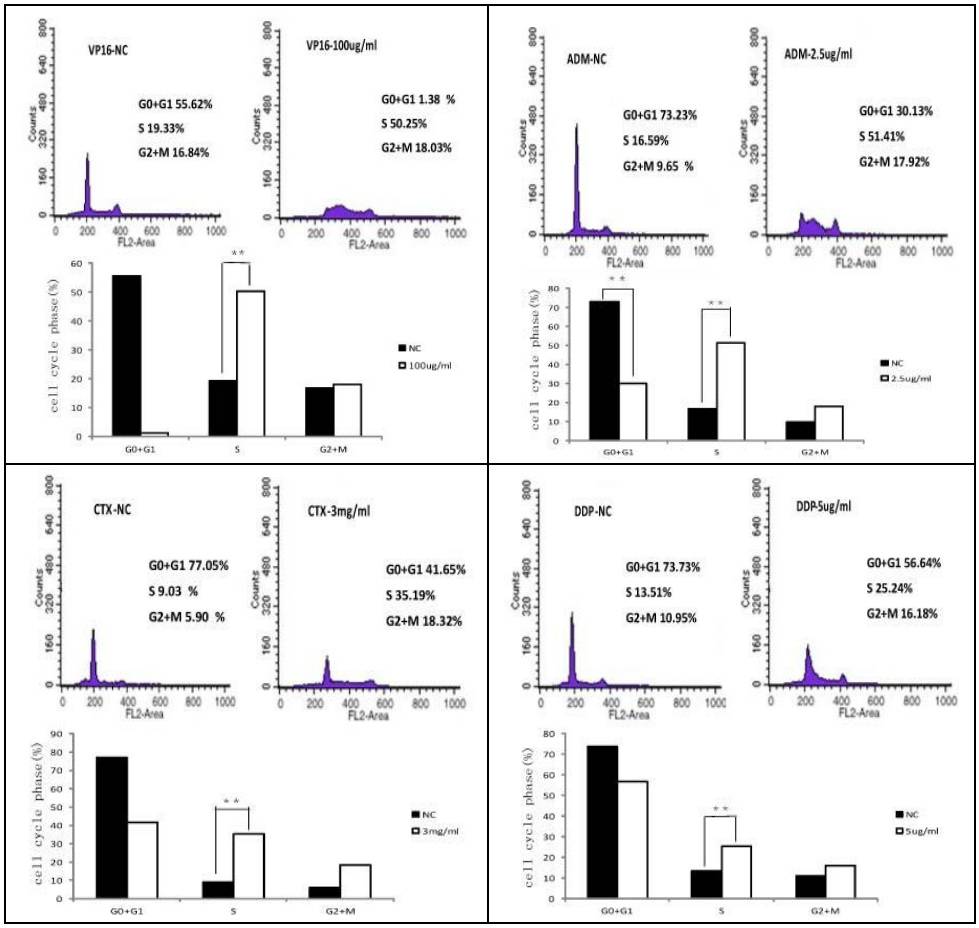

Figure 1. The influences of ADM、CTX、VP16 and DDP on SK-N-SH cell cycle.

*A: ADM group, Change of G0/G1 phase between control group and 2.5μg/ml group has significances difference. B CTX group: number of cells in G0/G1 phase between control group and 2.5μg/ml group has significances difference, C: VP16 group: Change of G0/G1 phase between control group and 2.5μg/ml group has significances difference, D: DDP group. ** P<0.01: Change of G0/G1 phase between control group and 2.5μg/ml group has significances difference.*

4. Discussions

The eukaryotic cell cycle is a very sophisticated programmed process. It contains G1 phase, S phase, G2 and M phases. The S phase and G2 is cell proliferation phase, until the M phase cells mitosis were ending [11]. G1 phase is the phase of RNA and ribosome synthetic period, as well as the period of external factors such as chemotherapy drugs working time. Loss of cells in the G1 phase can inhibit DNA synthesis and cells can't go into S phase, blocking S period to the G2/M phase transformation, and resulting in G2/M phase cells increased relatively. As a form of cell apoptosis, G2/M phase cells increased is the general phenomenon of cell damage. Cell cycle arrest and apoptosis were closely associated with the chemotherapy sensitivity of tumor cells. After arresting, the cell apoptosis can be accelerated. Therefore, the process of cell cycle arrest can promote apoptosis of tumor and likely to enhance the chemotherapy sensitivity of tumor cells. It has important significance for guiding clinical chemotherapy by studying tumor cell cycle regulatory mechanism [12].

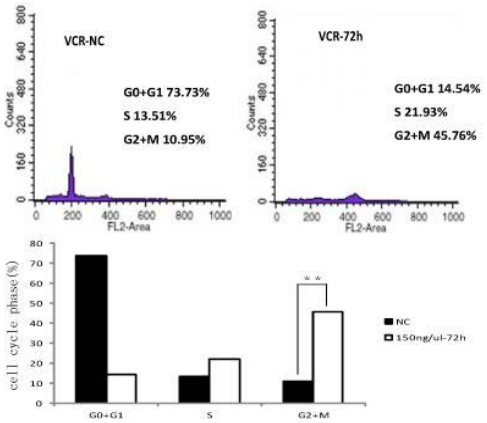

Figure 2. The influences of VCR on SK-N-SH cell cycle, ** P＜0.01.

Meirelles [13] reported that pyrimidine metabolic drugs such as ADM can induce cancer cells blocking in S period, and with the increase of the ADM concentration and action time extended, the number of cells block in G0/G1 phase increased. Zhong [14] reported that VCR can block the cancer cells such as chronic lymphocytic leukemia cells, human prostate cancer cells PC-3, thymus cancer cells and ovarian cancer cells in the G2/M phase. Peng [15] confirmed that DDP can pull ovarian cancer cell line HO-8910 down in the G1 phase of the cell cycle, and increase in S phase and G2/M phase. Prochazka [16] found that with the action of corn ketene, matrine and DDP on NB cells cell growth was observed at a slower pace, while Go/G1 phase cells proportion increase and S phase cell proportion reduced. The cell cycle was blocked in the Go/G1 phase. The research results show that different chemotherapy drugs may have different impact on the cell cycle, and this influence is likely to be due to cell cycle checkpoint pathways activated.

Our results show that: with the method of flow cytometry analysis, cells of SK-N-SH has obvious killing effect by ADM, and it makes the cell cycle block in G0/G1 phase, and Meirelles [13] also had the consistent conclusions. VCR can make NB cell cycle obviously block in G2/M phase. The research conclusions were also same with Zhong [14]. In this experiment, we found that the influence of DDP to SK-N-SH was block in G0/G1 phase [16]. This may be related to difference species of NB cell lines, different condition of the tumor cells growth and different chemotherapy drug concentration and time selection or other factors. Our study also found that CTX and VP16 also can make the SK-N-SH cells cell cycle obviously block in G0/G1 phase. Five kinds of chemotherapy drugs have obvious effect to cell cycle, and the influence may be different to drugs.

The influence of different chemotherapy drugs on NB cell cycle has obvious differences. Studies suggest that the cell cycle protein is the most important targets in the process of regulating the cell cycle. It can further activate the genes which were associated with cycle signal pathways. As Trk family important signal system, MAPK signal pathway is mediating cell proliferation and differentiation. The phosphorylation of ERK pathway is mainly mediated cell proliferation and differentiation by promoting Rb protein phosphorylation when cells enter the stage of Gl and M [16, 17]. Cane [18] found that 2, 3-indole ketone can induced tumor cell death by inhibiting phosphorylation of ERK pathway. Another study found that differentiation RA can effectively cut down expression of c–myc and c-the fos oncogene activity, and inhibit cell proliferation. It can also raise the expression of p27 and p53, and block cell cycle in SK-N-SH Go/G1 phase, and then induce the NB cells to differentiate into mature [19-20]. So, different varieties of chemotherapy drugs can activate cell cycle protein, further affects signal pathways. Then the cell cycle arrest at different times. Cell cycle regulation has an important role in tumor killing mechanism.

Reference

[1] Han W, Wang HM. Refractory diarrhea: A paraneoplastic syndrome of neuroblastoma[J]. World J Gastroenterol, 2015, 21(25):7929-7932.

[2] Bagatell R, Cohn SL. Genetic discoveries and treatment advances in neuroblastoma[J]. Curr. Opin. Pediatr, 2016, 28(1):19-25.

[3] Peinemann F, van Dalen EC, Berthold F. Rapid COJEC induction therapy for high-risk neuroblastoma patients-cochrane review[J]. Klin Padiatr, 2016, 228(3):130-134.

[4] Chaturvedi NK, McGuire TR, Coulter DW, et al. Improved therapy for neuroblastoma using a combination approach: superior efficacy with vismodegib and topotecan[J]. Oncotarget, 2016, 7(12):15215-15229.

[5] Cheung BB. Combination therapies improve the anticancer activities of retinoids in neuroblastoma[J]. World J. Clin. Oncol, 2015, 6(6):212-215.

[6] Elborai Y, Hafez H, Moussa EA, et al. Comparison of toxicity following different conditioning regimens (busulfan/melphalan and carboplatin/etoposide/melphalan) for advanced stage neuroblastoma: Experience of two transplant centers[J]. Pediatr Transplant, 2016, 20(2):284-289.

[7] Sawada A, Shimizu M, Isaka K, et al. Feasibility of HLA-haploidentical hematopoietic stem cell transplantation with post-transplantation cyclophosphamide for advanced pediatric malignancies[J]. Pediatr. Hematol. Oncol., 2014, 31(8):754-764.

[8] Ahmadi F, Mojarrab M, Ghazi-Khansari M, et al. A semipolar fraction of petroleum ether extract of Artemisia aucheri induces apoptosis and enhances the apoptotic response to doxorubicin in human neuroblastoma SKNMC cell line[J]. Res. Pharm. Sci., 2015, 10(4):335-344.

[9] Campos-Arroyo D, Maldonado V, Bahena I, et al. Probenecid sensitizes neuroblastoma cancer stem cells to cisplatin[J]. Cancer Invest, 2016, 34(3):155-166.

[10] Zhang D, Jia J, Zhao G, et al. NDRG1 promotes the multidrug resistance of neuroblastoma cells with upregulated expression of drug resistant proteins[J]. Biomed. Pharmacother, 2015, 76:46-51.

[11] Chen J. The cell-cycle arrest and apoptotic functions of p53 in tumor initiation and progression[J]. Cold Spring Harb Perspect Med., 2016, 6(3):a026104.

[12] Setiawati A, Setiawati A. Celecoxib, a cox-2 selective inhibitor, induces cell cycle arrest at

the g2/m phase in hela cervical cancer cells[J]. Asian Pac. J. Cancer Prev., 2016, 17(4):1655-60.
[13] Meirelles K, Benedict LA, Dombkowski D, et al. Human ovarian cancer stem/progenitor cells are stimulated by doxorubicin but inhibited by Mullerian inhibiting substance[J]. Proc. Natl. Acad. Sci. USA., 2012, 109(7):2358-2363.
[14] Zhong YJ, Shi F, Zheng XL, et al. Crocetin induces cytotoxicity and enhances vincristine-induced cancer cell death via p53-dependent and -independent mechanisms[J]. Acta. Pharmacol. Sin., 2011, 32(12):1529-1536.
[15] Peng LP, Huang JM, Zhang GN, et al. Influence of human epithelial ovarian cancer HO-8910 cells with modified survivin gene on the cell cycle distribution and chemosensitivity[J]. Zhonghua Fu Chan Ke Za Zhi. 2010, 45(11):860-864.
[16] Prochazka P, Hrabeta J, V ćha A, et al. Expulsion of amplified MYCN from homogenously staining chromosomal regions in neuroblastoma cell lines after cultivation with cisplatin, doxorubicin, hydroxyurea, and vincristine[J]. Cancer Genet Cytogenet, 2010, 196(1):96-104.
[17] Bettayeb K, Oumata N, Echalier A, et al. CR8, a potent and selective, roscovitine-derived inhibitor of cyclin-dependent kinases[J]. Oncogene, 2008, 27(44):5797-5807.
[18] Ploessl C, Pan A, Maples KT, et al. Dinutuximab: An anti-gd2 monoclonal antibody for high-risk neuroblastoma[J]. Ann Pharmacother, 2016, 50(5):416-422.
[19] Cane G, Moal VL, Pag ès G, et al. Up-regulation of intestinal vascular endothelial growth factor by Afa/Dr diffusely adhering Escherichia coli[J]. PLoS One, 2007, 2(12):e1359.
[20] Maugeri G, D'Amico AG, Ras à DM, et al. Expression profile of Wilms Tumor 1 (WT1) isoforms in undifferentiated and all-trans retinoic acid differentiated neuroblastoma cells[J]. Genes Cancer, 2016, 7(1-2):47-58.

