European Review for Medical and Pharmacological Sciences 2017; 21: 612-617

Arsenic trioxide regulates gastric cancer cell apoptosis by mediating cAMP

A. ABUDOUREYIMU[1], A. MUHEMAITIBAKE[2]

[1]Department of Geriatrics, Xinjiang Medical University Affiliated Hospital of Traditional Chinese Medicine, Urumqi, Xinjiang, China
[2]Dashizi Outpatient Departmens, Xinjiang Medical University Affiliated Hospital of Traditional Chinese Medicine, Urumqi, Xinjiang, China

Abstract. – OBJECTIVE: Gastric cancer is a common digestive tract tumor in clinic with increasing incidence. It is suggested that arsenic trioxide (As_2O_3) has an inhibitory effect on many kinds of digestive system tumors. This study evaluated the impact of As_2O_3 on the apoptosis of gastric cancer BGC-823 cells, and analyzed its relationship with cyclic adenosine monophosphate (cAMP).

MATERIALS AND METHODS: Gastric cancer cell BGC-823 was intervened by different concentrations of As_2O_3 at 4 ng/ml, 8 ng/ml, and 16 ng/ml, respectively. BGC-823 cell apoptosis was evaluated by TUNEL assay. Cell cycle was determined by flow cytometry. cAMP and protein kinase C (PKC) was detected by radioimmunoassay. Apoptosis-related protein levels were tested by Western blot.

RESULTS: Compared with normal control, As2O3 significantly increased BGC-823 cell apoptosis, blocked cell cycle in G0/G1 phase, elevated cAMP and Bax level, as well as downregulated PKC, Bcl-2 and Survivin expression ($p < 0.05$).

CONCLUSIONS: As_2O_3 induced BGC-823 cell apoptosis through the up-regulation of the cAMP level and the decrease of the PKC level.

Key Words:
As_2O_3, Gastric cancer, Apoptosis, cAMP, PKC.

Introduction

Unlimited proliferation is characteristic of cancer cells. Except for number increase, they are also featured as apoptosis restriction[1,2]. Arsenic trioxide, also known as As_2O_3, is previously used in the treatment of acute myeloid leukemia. A recent study[3] revealed that As_2O_3 has the inhibitory effect on digestive system neoplasm by inducing cell apoptosis. It is found that the process of cell apoptosis is regulated by cytokines and signaling pathways, such as Ca^{2+}, cyclic adenosine monophosphate (cAMP), protein kinase C (PKC), and Caspase signaling pathways[4,5]. The cAMP is an important second messenger involved in cell proliferation, division, apoptosis, and other life activities[6]. PKC is a Ca^{2+} and phospholipids dependent protein kinase that can regulate cell apoptosis process. PKC elevation may suppress cell apoptosis, while PKC downregulation increases cell apoptosis rate[7]. This study selected gastric cancer BGC-823 cell and intervened it with different concentrations of As_2O_3 to analyze the effect of As_2O_3 on BGC-823 cell apoptosis.

Materials and Methods

Experimental Cells

Gastric cancer cell line BGC-823 was provided by Xinjiang Medical University Culture Center (Urumqi, Xinjiang, China).

Reagents and Instruments

As_2O_3 was from ProSpec (ProSpec, East Brunswick, NJ, USA). RPMI-1640, penicillin, and streptomycin were provided by Gibco (Thermo Fisher Scientific, Waltham, MA, USA). The cAMP kit and PKC kit were got from Gibco BRL (Thermo Fisher Scientific, Waltham, MA, USA). The centrifuge was from Beckman (Beckman Coulter, Brea, CA, USA).

Experimental Methods

Conventional Cell Culture

BGC-823 cells were cultured in RPMI-1640 medium and maintained in 37°C and 5% CO_2.

Corresponding Author: Ayinuer Muhemaitibakeo, MD; e-mail: HongyuZhengzxc@163.com

As_2O_3 Intervention

BGC-823 cells in logarithmic phase were seeded in a plate and treated with different concentrations of As_2O_3 at 4 ng/ml, 8 ng/ml, and 16 ng/ml, respectively.

TUNEL Assay

After treated by As_2O_3, BGC-823 cells were washed with PBS and added with TdT enzyme (Merck, Temecula, CA, USA). After added by the anti-digoxin antibody (Abcam, Cambridge, MA, USA), the cells were developed by DAB.

Cell Cycle Detection

BGC-823 cells in logarithmic phase were centrifuged and fixed at 4°C overnight. After treated by PI avoid of light for 30 min, the cells were detected by flow cytometry (BD, San Jose, CA, USA).

Radio Immunoassay

The cells were digested and treated by RIPA (Merck, Temecula, CA, USA) at 0°C for 30 min. After centrifugation, the supernatant was added with 3H-cAMP and 32P-substrate (Merck, Temecula, CA, USA). After centrifugation, the supernatant was treated with scintillation solution to test radioactivity according to the manual.

Western Blot

Total protein was extracted from the cells and separated by SDS-PAGE. After blocked at RT, the membrane was incubated in primary antibody (1:200, -actin 1:500) and secondary antibody (1:2000) (Abcam, Cambridge, MA, USA) at RT for 1 h in sequence. After developed by buffer A and B, the membrane was read to analyze the relative protein expression.

Statistical Analysis

SPSS 17.0 software (IBM, Armonk, NY, USA) was applied for data analysis. The data was presented as mean ± standard deviation. Enumeration data was compared by χ^2-test, while measurement data was compared by *t*-test. $p < 0.05$ was considered as statistical significance.

Results

As_2O_3 Induced BGC-823 Cell Apoptosis

After treated by As_2O_3, BGC-823 cell apoptosis was evaluated by TUNEL assay. Light gray staining was observed in BGC-823 cells of the control group, while deep brown staining was shown in that of the experimental group. Cell apoptosis increased in the experimental group compared with that in the control group, which was in a dose dependence manner following the addition of As_2O_3 ($p < 0.05$) (Figures 1 and 2).

As_2O_3 Blocked BGC-823 Cell Cycle

Different concentrations of As_2O_3 were applied in the treatment of BGC-823 cells for 48 h. Flow cytometry was performed to test cell cycle. With the elevating dose of As_2O_3, the amount of cells in G0/G1 phase gradually increased, while that in S and G2/M phases declined ($p < 0.05$) (Figures 3 and 4).

As_2O_3 Affected cAMP and PKC Levels in BGC-823 Cells

cAMP and PKC levels in BGC-823 cells were tested by radioimmunoassay. The result demonstrated that As_2O_3 markedly upregulated cAMP level, while reduced PKC level in BGC-823 in a dose dependent manner ($p < 0.05$) (Figure 5).

As_2O_3 Affected Bcl-2, Bax, and Survivin Protein Expressions in BGC-823 Cells

Western blot was performed to determine Bcl-2, Bax, and Survivin protein expression in BGC-823 cells after the treatment of As_2O_3. It was found that levels of Bcl-2 and Survivin declined, whereas Bax expression enhanced in the experimental group following the increasing level of As_2O_3 concentration (Table I, Figure 6).

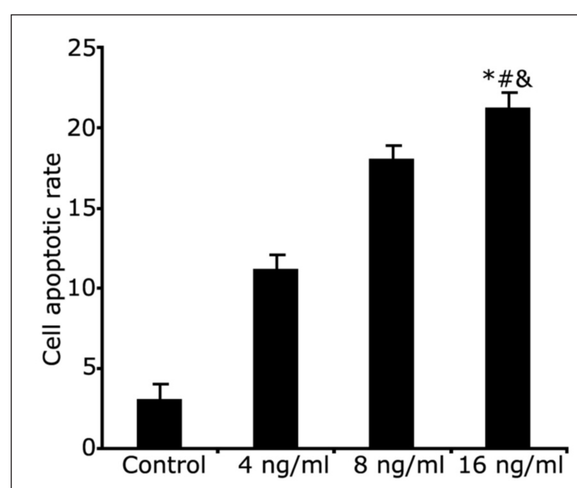

Figure 1. BGC-823 cell apoptosis analysis. $^*p < 0.05$, compared with normal control. $^\#p < 0.05$, compared with 4 ng/ml group. $^\&p < 0.05$, compared with 8 ng/ml group.

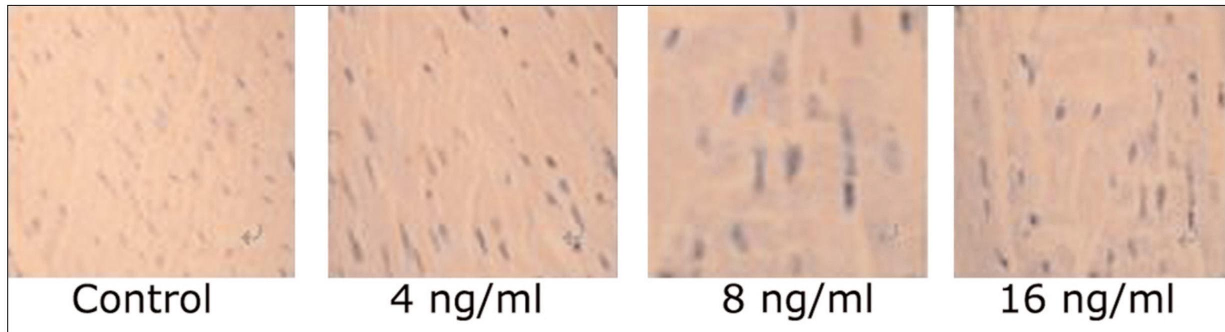

Figure 2. As_2O_3 induced BGC-823 cell apoptosis.

Discussion

The formation of the malignant tumor is caused by malignant tumor cell unlimited proliferation, leading to an imbalance between cell survival and death. Cell number unlimited increase eventually forms the malignant tumor[8,9]. It was reported that As_2O_3 inhibited DNA and RNA synthesis process, resulting in chromosome mutation[10]. In this study, gastric cancer BGC-823 cells were treated with different concentrations of As_2O_3 to analyze its mechanism on apoptosis.

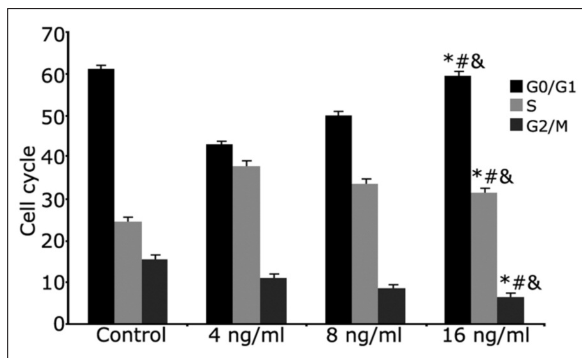

Figure 3. BGC-823 cell cycle analysis. $^{*}p < 0.05$, compared with normal control. $^{\#}p < 0.05$, compared with 4 ng/ml group. $^{\&}p < 0.05$, compared with 8 ng/ml group.

TUNEL assay showed that the cells in the experimental group presented deep brown staining under a microscope. The cell apoptosis rate significantly increased in experimental group compared with control, and the rate was gradually upregulated following the rise of As_2O_3 concentration. It's suggested that As_2O_3 can induce gastric cancer BGC-823 cell apoptosis. It was reported that low concentration of As_2O_3 induced cancer cell apoptosis to suppress tumor growth[11]. Our result also was consistent with previous finding that As_2O_3caused thiol enzyme inactivation, block tumor cell cycle, inhibit tumor cell proliferation, and further induce tumor cell differentiation and apoptosis[12].

Cell cycle analysis revealed that cell rate in G0/G1 phase markedly increased, while in S and G2/M phases declined in BGC-823 cells treated by As_2O_3 in a dose-dependent manner. It

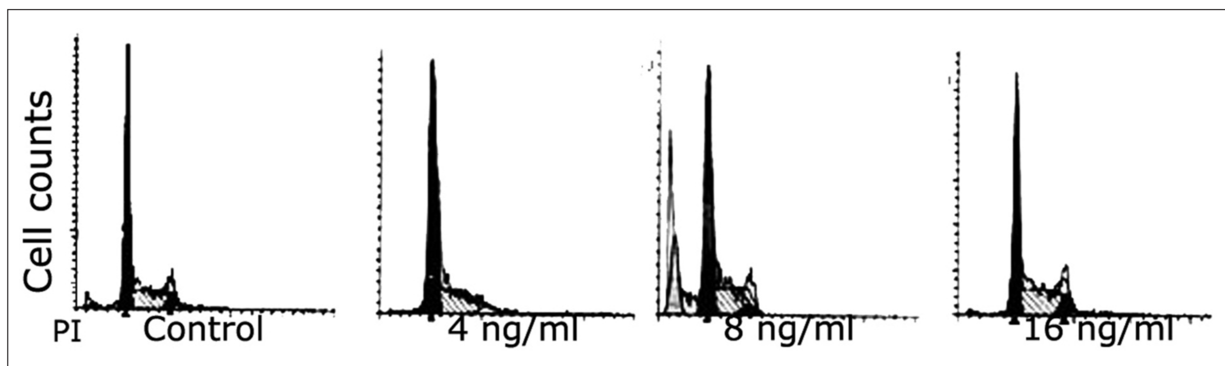

Figure 4. As_2O_3 blocked BGC-823 cell cycle.

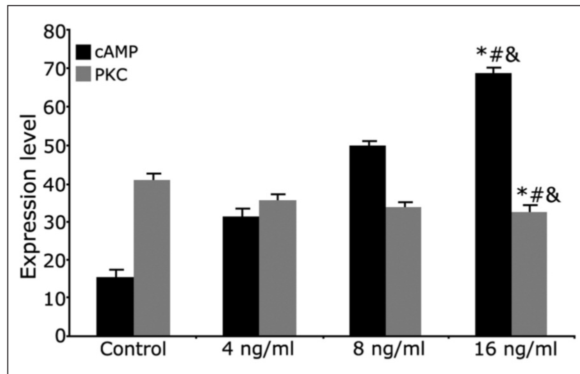

Figure 5. cAMP and PKC levels in GBC-823 cells. $^{*}p < 0.05$, compared with normal control. $^{\#}p < 0.05$, compared with 4 ng/ml group. $^{\&}p < 0.05$, compared with 8 ng/ml group.

indicated that As_2O_3 blocked BGC-823 cells in G0/G1 phase, thus to restrain cells entering S phase and suppress cell proliferation. Previous research proved that As_2O_3 could induce gastric cancer cell apoptosis and restrain cell cycle, resulting in gastric cancer cell necrosis and apoptosis and thus inhibiting cancer progress[13,14]. cAMP, mainly activating protein kinase A, is found to affect the endogenous endonuclease activity to achieve the purpose of apoptosis induction[15,16]. PKC is a kind of protein kinase that affects signaling pathway and is involved in various physiological and pathological processes, such as cell metabolism, differentiation, and proliferation[17,18]. Previous researches[19,20] detected PKC and cAMP levels in the cells and found that PKC downregulation and cAMP elevation obviously suppressed cell proliferation and enhanced cell apoptosis. Our work demonstrated that As_2O_3 upregulated cAMP level and reduced PKC level in BGC-823 cells. It was proved that cAMP analogue or cAMP inducer significantly elevated cAMP concentration in the cells, which thus induced cell apoptosis. The induction effect of cAMP on cells may be mediated by both PKA and cAMP dependent protein kinase I, which in turn activated corresponding response element to exert its biological function. It seems that as a differentiation inducer, the reduction of cAMP causes canceration. From the perspective of treatment, rebuilding the level of cAMP in the cells might be an effective strategy to inhibit the progression of the tumor. Our findings showed that As_2O_3 enhanced the cAMP level and declined PKC level in BGC-823 cells, which was proposed to be one of the mechanisms of promoting cell apoptosis.

Finally, we adopted Western blot to test the apoptosis-related proteins expression in BGC-823 cells, including the detection of Bcl-2, Bax, and Survivin. As_2O_3 treatment decreased Bcl-2 and Survivin levels, and enhanced Bax expression. Bcl-2 has been confirmed to be negatively correlated with apoptosis, while shows no effect on mitosis. As an important apoptosis suppressor, Bcl-2 is proved to reduce Bax expression level, thus to suppress apoptosis through a series of signaling pathways. Survivin is the hotspot of antitumor treatment in clinic by involving in the regulation of cell apoptosis and regulating mitosis. This study suggested that As_2O_3 induced cell apoptosis through downregulating Bcl-2 and Survivin expression while enhancing Bax level in BGC-823 cells.

Conclusions

As_2O_3 induced gastric cancer cell apoptosis by blocking BGC-823 cells in G0/G1 phase, elevating cAMP level, reducing PKC level, downregulating Bcl-2 and Survivin expression, and enhancing Bax level. It may be treated as new leads for the discovery of As_2O_3 therapy on gastric cancer. Further in-depth investigation needed to clarify the specific mechanism.

Table I. Bcl-2, Bax, and Survivin protein expression analysis in BGC-823 cells treated by As_2O_3.

Group	Bcl-2	Bax	Survivin
Experiment			
4 ng/ml	$0.53 \pm 0.13^{*}$	$0.16 \pm 0.09^{*}$	$0.53 \pm 0.21^{*}$
8 ng/ml	$0.27 \pm 0.08^{*\#}$	$0.39 \pm 0.11^{*\#}$	$0.29 \pm 0.12^{*\#}$
16 ng/ml	$0.14 \pm 0.01^{*\#\&}$	$0.53 \pm 0.37^{*\#\&}$	$0.18 \pm 0.07^{*\#\&}$
Control	0.89 ± 0.41	0.81 ± 0.57	0.72 ± 0.47

$^{*}p < 0.05$, compared with normal control. $^{\#}p < 0.05$, compared with 4 ng/ml group. $^{\&}p < 0.05$, compared with 8 ng/ml group.

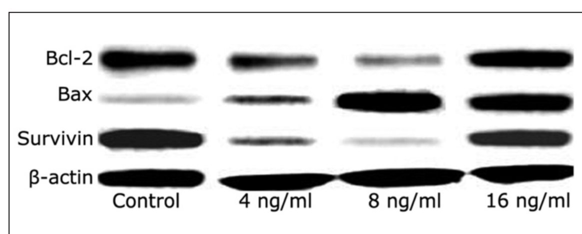

Figure 6. As_2O_3 affected Bcl-2, Bax, and Survivin protein expressions in BGC-823 cells.

Acknowledgements

This work was supported by Research project of combining traditional Chinese medicine and Western medicine with traditional Chinese medicine in the Xinjiang Uygur Autonomous Region (No. 20160403).

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Arababadi MK Asadikaram G. Opium induces apoptosis in Jurkat cells via promotion of pro-apoptotic and inhibition of anti-apoptotic molecules. Iran J Basic Med Sci 2016; 19: 215-220.
2) Campbell TL, Quadrilatero J. Data on skeletal muscle apoptosis, autophagy, and morphology in mice treated with doxorubicin. Data Brief 2016; 7: 786-793.
3) Slejkovec Z, Podgornik H, Cernelc P, Falnoga I. Exceptions in patterns of arsenic compounds in urine of acute promyelocytic leukaemia patients treated with As_2O_3. Biometals 2016; 29: 107-118.
4) Borthwick LA, Kerbiriou M, Taylor CJ, Cozza G, Lascu I, Postel EH, Cassidy D, Trouve P, Metha A, Robson L, Muimo R. Role of interaction and nucleoside diphosphate kinase b in regulation of the cystic fibrosis transmembrane conductance regulator function by cAMP-dependent protein kinase A. PLoS One 2016; 11: e0149097.
5) Hu S, Cao Q, Xu P, Ji W, Wang G, Zhang Y. Rolipram stimulates angiogenesis and attenuates neuronal apoptosis through the cAMP/cAMP-responsive element binding protein pathway following ischemic stroke in rats. Exp Ther Med 2016; 11: 1005-1010.
6) Takeda Y. Theoretical investigations into the quantitative mechanisms underlying the regulation of [cAMP]i, membrane excitability and [Ca(2+)]i during GLP-1 stimulation in pancreatic beta cells. Yakugaku Zasshi 2016; 136: 467-471.
7) Arya A, Gangwar A, Singh SK, Roy M, Das M, Sethy NK, Bhargava K. Cerium oxide nanoparticles promote neurogenesis and abrogate hypoxia-induced memory impairment through AMPK-PKC-CBP signaling cascade. Int J Nanomedicine 2016; 11: 1159-1173.
8) Genov M, Kreiseder B, Nagl M, Drucker E, Wiederstein M, Muellauer B, Krebs J, Grohmann T, Pretsch D, Baumann K, Bacher M, Pretsch A, Wiesner C. Tetrahydroanthraquinone derivative (+/-)-4-deoxyaustrocortilutein induces cell cycle arrest and apoptosis in melanoma cells via upregulation of p21 and p53 and downregulation of NF-kappaB. J Cancer 2016; 7: 555-568.
9) Yousef BA, Hassan HM, Guerram M, Hamdi AM, Wang B, Zhang LY, Jiang ZZ. Pristimerin inhibits proliferation, migration and invasion, and induces apoptosis in HCT-116 colorectal cancer cells. Biomed Pharmacother 2016; 79: 112-119.
10) Erturk FA, Aydin M, Sigmaz B, Taspinar MS, Arslan E, Agar G, Yagci S. Effects of As_2O_3 on DNA methylation, genomic instability, and LTR retrotransposon polymorphism in Zea mays. Environ Sci Pollut Res Int 2015; 22: 18601-18606.
11) Chen P, Wu J, Yuan Q, Jiang X, Huang H. The synergistic killing of AML cells co-cultured with HS-5 bone marrow stromal cells by As2O3 and the PI3K/Akt signaling pathway inhibitor LY294002. Pharmazie 2015; 70: 322-327.
12) Pastorek M, Gronesova P, Cholujova D, Hunakova L, Bujnakova Z, Balaz P, Duraj J, Lee TC, Sedlak J. Realgar (As_4S_4) nanoparticles and arsenic trioxide (As_2O_3) induced autophagy and apoptosis in human melanoma cells in vitro. Neoplasma 2014; 61: 700-709.
13) Garcia-Sevillano MA, Garcia-Barrera T, Navarro F, Gomez-Ariza JL. Analysis of the biological response of mouse liver (Mus musculus) exposed to As2O3 based on integrated -omics approaches. Metallomics 2013; 5: 1644-1655.
14) Petit A, Delaune A, Falluel-Morel A, Goulle JP, Vannier JP, Dubus I, Vasse M. Importance of ERK activation in As_2O_3-induced differentiation and promyelocytic leukemia nuclear bodies formation in neuroblastoma cells. Pharmacol Res 2013; 77: 11-21.
15) Raker VK, Becker C, Steinbrink K. The cAMP pathway as therapeutic target in autoimmune and inflammatory diseases. Front Immunol 2016; 7: 123.
16) Nakamura T, Kamishikiryo J, Morita T. Prazosin-stimulated release of hepatic triacylglyceride lipase from primary cultured rat hepatocytes is involved in the regulation of cAMP-dependent protein kinase through activation of the Ca(2+)/calmodulin-dependent protein kinase-II. Pharmacol Rep 2016; 68: 649-653.
17) Zhang F, Domg W, Zeng W, Zhang L, Zhang C, Qiu Y, Wang L, Yin X, Zhang C, Liang W. Naringenin prevents TGF-beta1 secretion from breast cancer and suppresses pulmonary metastasis by inhibiting PKC activation. Breast Cancer Res 2016; 18: 38.

18) Lee YY, Ryu MS, Kim HS, Suganuma M, Song KY, Lim IK. Regulations of reversal of senescence by pkc isozymes in response to 12-O-Tetradecanoylphorbol-13-Acetate via Nuclear Translocation of pErk1/2. Mol Cells 2016; 39: 266-279.

19) Lin P, Cheng T, Ma S, Gao J, Jin S, Jiang L, Xia Q. Bacillus bombysepticus alpha-toxin binding to G protein-coupled receptor kinase 2 regulates cAMP/PKA signaling pathway to induce host death. PLoS Pathog 2016; 12: e1005527.

20) Peng R, Zhao GX, Li J, Zhang Y, Shen XZ, Wang JY, Sun JY. Auphen and dibutyryl cAMP suppress growth of hepatocellular carcinoma by regulating expression of aquaporins 3 and 9 in vivo. World J Gastroenterol 2016; 22: 3341-3354.