J. Exp. Biomed. Sci. 12 (2006) 153-160

The Effect of Taxol and Arsenic Trioxide in HT-29 Spheroid Cells

InSoo Lee, HyunIl Choi, HyeEun Han, HyeYoung Lee and TaeUe Kim†

Department of Biomedical Laboratory Science, College of Health Science, Yonsei University, Wonju 220-710, Korea

Human colon cancer is the second most fatal disease among a variety of cancers to cause cancer death in U.S.A. and its incidence rate is currently increased in Korea. Recently, many studies have been being progressed on the efficacy of diverse combination treatments. But results of these studies *in vitro* were not similar those *in vivo*. This study compared the anticancer reactions between each use of arsenic trioxide and taxol against human colon cancer HT-29 cell line and combined use of two drugs. And these results compared with the results of HT-29 spheroid cells having similar characteristics to the solid tumor *in vivo*. The spheroid of HT-29 cells was formed by using a multicellular spheroid system and the result was observed through electron microscopy. *In vitro* cytotoxicity of each use of arsenic trioxide and taxol was evaluated in HT-29 monolayer cells. The IC_{50} value for arsenic trioxide was to be 33 μM and taxol was to be 18 nM. The result treated with the combination of taxol and arsenic trioxide decreased the cytotoxicity on the HT-29 monolayer cells. The spheroid cells represented higher resistance against drugs than the monolayer cells. I demonstrated DNA fragmentation after incubation with concentrations more than 10 μM arsenic trioxide and 100 nM taxol for 48 h on the monolayer cells. But the results of HT-29 cell line treated with the combination of taxol and arsenic trioxide was the same as the outcome of control samples that were not treated with any drug. And I don't demonstrated DNA fragmentation on the spheroid cells. These results suggest that apoptosis was not induced in the use of the combination can be thought as that arsenic trioxide might work as an antagonist to inhibit a taxol mechanism to induce apoptosis. And the spheroid cells represented higher resistance against drugs than the monolayer cells.

Key Words: Taxol, Arsenic trioxide, Apoptosis, HT-29 cell line, Monolayer, Spheroid, Antagonism

INTRODUCTION

As recently Korea has developed into an advanced country, the people have eaten more meat and animal fat, which has contributed greatly to the rapidly increasing incidence of colorectal cancer in Korea. According to its National Cancer Statistical Survey in, the incidence of colorectal cancer was ranked fourth in both the males and the females; cases with the cancer accounted for no less than 10.3% of the entire cancer patients and are steadily increasing over time; and the resulting death rate was estimated at approximately 8.9 per a hundred thousand patients. For the purpose of cancer treatment, many useful anticancer drugs have been discovered by picking up synthetic derivatives or natural materials for a long time. However, because it is impossible to treat most of advanced tumors with chemical therapies, developing a new anticancer drug became weighty in confrontation with cancer nowadays. Experimental studies on anticancer agent susceptibility are performed in the two aspects of randomized screening and combination with well-known anticancer drugs. The National Cancer Institute abbreviated to NCI (U.S.A.) has used sixty kinds of cell line panels - customized specially for the types of disease originated from lung cancer, colorectal cancer, breast cancer, prostate cancer, ovarian cancer, kidney cancer, brain tumor, etc. - in selecting a primary anticancer agent (Jiang, 1986). However, outcomes on solid cancers don't show homeostasis. Actually, such monolayer cells cultured *ex vivo* are completely identical with the tissues in the body. Even if the growth physiology of cells and intercellular connections appear similar in the monolayer cell-culturing method,

*Received: August 20, 2006
Accepted after revision: September 7, 2006
†Corresponding author: TaeUe Kim, Department of Biomedical Laboratory Science, College of Health Science, Yonsei University, Wonju 220-710, Korea.
Tel: 82-33-760-2424, Fax: 82-33-760-2195
e-mail: kimtu@yonsei.ac.kr

the cultured cells are characterized not by three-dimensional structure like that of solid cancer, but by two-dimensional one. Furthermore, it is never possible to imitate the unique structure and heterogeneous natures of cancer by means of cultured monolayer cells. Hence, it cannot be expected to evaluate correctly the effects of anticancer drugs founded as the result of *in-vivo* experiments in special cases, for example, ischemic solid cancer where it is considered that several factors, such as oxygen tension, extracellular acidity (pH), nutrients and metabolites, to affect the effects of chemotherapy and radiotherapy.

In order to overcome such the disadvantage, some experimental models intended to represent the three-dimensional characteristics of cancer were developed, for instance, collagen gel culture, mesh-supported organoid histoculture or multicellular spheroid culture. These models are expected to generate similar conditions to those of cancer and establish an important basis for drug identification designed to treat solid cancers due to the type of histoculture based on spherical cellular overlapping. Of them, spheroid cell culture is different from collagen-gel culture (Chiung-Tong et al., 1997) in which cell supernatant is spreaded onto collagen-gel and histoculture (Hirano et al., 1999; Hirano et al., 2003) in which the supernatant is embedded in the sponge matrix containing either cellulose or collagen. For spheroid cell culture, cellular monolayer is not formed by maintaining constant spinning without any matrix and cells grow up densely into a sort of spherical form (Kobayashi et al., 1993; Wartenberg et al., 2001; Sonoda et al., 2003). Despite of their improvements, these sophisticated culture methods also have the critical disadvantage of demanding techniques and long time required for such culture. For the reason, they cannot be used as a universal selection method. However, they have been used restrictively for some special purposes of research or as a model for secondary selection. Particularly, spheroid cell culture has been used widely as a model intended for drug selection and search for culture conditions.

Taxol is a natural substance isolated from *Taxaceae* tree, *Taxus brevifolia* which is known very efficacious against ovarian cancer and breast cancer as well as even other cancer cells (Rowinsky, 1995). It is known that taxol inhibits cellular differentiation (Jordan et al., 1993) and induce cells to die (Lopes et al., 1993; Bhall et al., 1993; Milas et al., 1995); concretely speaking, its efficacy and effectiveness are obtained in the manner of combining directly with microtubules, inhibiting their degradation to stabilize them, and suppressing cell division at the phase G2/M of cell cycle (Rieder et al., 1994; Derry et al., 1995; Long et al., 1994).

The harmfulness of arsenic trioxide (As_2O_3) was already ascertained long before and is well-known at present; if high-dose arsenic trioxide is taken in the body, highly fatal result may occur, and repeated or constant exposure to arsenic trioxide may lead to nervous system disorders, liver diseases or peripheral vascular diseases, increasing substantially the risk of cancer (Gebel, 2000). On the other hand, a trace of arsenic trioxide has been employed as a traditional treatment in the Oriental medicine (Ratnaike, 2003), and it was revealed that a low level of arsenic trioxide (As_2O_3) in the blood is positively effective for treatment of acute promyelocytic leukemia (APL) (Soignet et al., 1998; Shen et al., 1997). A recent study reported that arsenic trioxide inhibits cellular growth depending upon its concentration in colorectal cancer cell lines including SW480, DLD-1, COLO201 and so on as well (Nakagawa et al., 2002; Huang et al., 2000).

Lately, a study is being conducted, in which several anticancer drugs are properly combined and administered so that significant anticancer effects are obtained against the cancers resistant to individual anticancer drugs (Carles et al., 1998; Fracasso et al., 2000). There are also ongoing studies on therapeutic effects of combined drugs having similar mechanisms of action and studies which expect any additional effect or synergy by using simultaneously several kinds of anticancer agents with different targets (Nabholtz et al., 2001; Kohn et al., 2001; Manon et al., 2002).

In this study, it was attempted to clarify whether the results from monolayer cell culture can apply to *in vivo* conditions in the manner of preparing similar spheroid cells to *in vivo* solid cancer and making a comparison with the findings in the HT-29 human colorectal cancer cells cultured into monolayer cells, for the purpose of investigate the effects of arsenic trioxide and taxol on colorectal cancer, a type of solid cancer when they are administered separately and in the combined form. Thus, this study involved culturing a colorectal cancer cell line, HT-29 into spheroid cells, treating taxol and arsenic trioxide under the same conditions as monolayer cell culture, and comparing the results, in order.

MATERIALS AND METHODS

1. Generation of multicellular tumor spheroids

HT-29 (Human colon cancer) cells were cultured at 37℃ in a 5% carbon dioxide atmosphere in RPMI-1640 medium (Gibco BRL, Grand Island, USA) supplemented with 10% heat-inactivated fetal bovine serum (Gibco BRL, Grand Island, USA) and antibiotics. Spheroids were obtained by inoculating 1.5×10^5 cells into the wells of a 48-well culture plate containing 300 μl of RPMI-FBS 10% on a layer of 200 μl of 1% (W/V) agar in PBS (pH 7.4).

2. Transmission electron microscope (TEM)

Spheroid cells were washed with PBS (pH 7.4) and resuspended in the same buffer. The cells that were to be processed for electron microscopy (JEM 1200 EX-II, JEOL, Japan) were centrifuged at 400 X *g*, fixed with 2.5% glutaraldehyde in PBS for 2 h, washed in 0.1 M Caocodylate (pH 7.4), and fixed with 0.1% OsO_4 in 0.1 M Caocodylate for 1.5 h. After fixation, the cells were washed with in 0.1 M Caocodylate, and then dehydrated in graded ethanol. Next, the cells were impregnated with propylene oxide and embedded in Polybed 812 (Polyscience, Inc., Warrington, USA). After incubation at 60℃, the cells were cut and stained with uranyl acetate and lead citrate.

3. Scanning electron microscope (SEM)

Spheroid cells were washed with phosphate solution (0.1 M, pH 7.4) and fixed in 2.5% cold glutaraldehyde in 0.1 M phosphate solution at 4℃ for 3 h. After washed three times, the samples were post-fixed in 1.0% osmium tetroxide (Fluka) for 2 h, pH 7.4. After dehydration with a series of ethanol gradient (25, 50, 75, 90 and 100%) for 10 min three times per gradient, the samples were dried in a critical point dryer and coated with gold on an ion sputter. They were examined under a scanning electron microscope (JEOL Co. Tokyo, Japan).

4. DNA extraction and electrophoresis

Samples were washed twice in a solution of 50 mM Tris-HCl, pH 8.0, 1 mM EDTA, 100 mM NaCl and were lysed with 500 μl lysis buffer (1% Triton X-100, 50 mM Tris-HCl, pH 7.5, 20 mM EDTA). Lysates were harvested by 1,000 X*g* for 10 min and the supernatants were incubated for 4 h at 37℃ with 50 μg/ml RNase A, 120 μg/ml proteinase K. Then phenol/chloroform/isoamylalcohol (25:24:1, Sigma) extracted the DNA. After precipitation, the pellets were resuspended in 30 μl TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). Each DNA sample was electrophoresed through 1.8% agarose gel contained with ethidium bromide. The gel was visualized by UV fluorescence.

5. Cell culture and *in vitro* cytotoxicity assay

HT-29 human colon cancer monolayer cells and spheroid cells were maintained in RPMI-1640 medium that was supplemented with 10% fetal bovine serum with 100 units/ml of penicillin, 100 μg/ml of streptomycin. All of the cells were grown at 37℃ in a humidified atmosphere of 5% CO_2. Cytotoxicity was measured by the microculture tetrazolium (MTT) method. Exponentially growing cells were inoculated to 1×10^4 cells/well using a 96 well microplate that was supplemented with 100 μl RPMI-1640. For experiments that studied cells in plateau phase of growth, the cells were permitted to grow for a minimum of 72 h before they were exposed to drugs. The cells were exposed to various concentrations of drug. After the treated cells were incubated for 24 h, 50 μl MTT (1 mg/ml, Sigma) was added and the plates were incubated at 37℃ for 4 h. To dissolve formazan, 100 μl DMSO was added and the plates were measured at 540 nm by spectrometer. The IC_{50} value was determined by plotting the drug concentration versus the survival ratio of the treated cells.

6. Frozen section and DAPI stain

Spheroid cells were washed with PBS (pH 7.4) and resuspended in the same buffer. The cells were fixed with 0.25% glutaraldehyde in PBS for 10 mins, and stained with 4', 6 diamidino-2-phenylindole (DAPI). The cells were placed in Tissue-Tek O.C.T. Compound (Sakura) and frozen in a mixture of dry ice and 2-propanol. Frozen sections, 8-μm thick, were prepared with a cryostatic ultramicrotome, fixed in 4% paraformaldehyde, and examined using fluorescence microscope and optical microscope.

RESULTS

1. Observation of spheroid cells by electron microscope

Usually, about three days are required to acquire complete

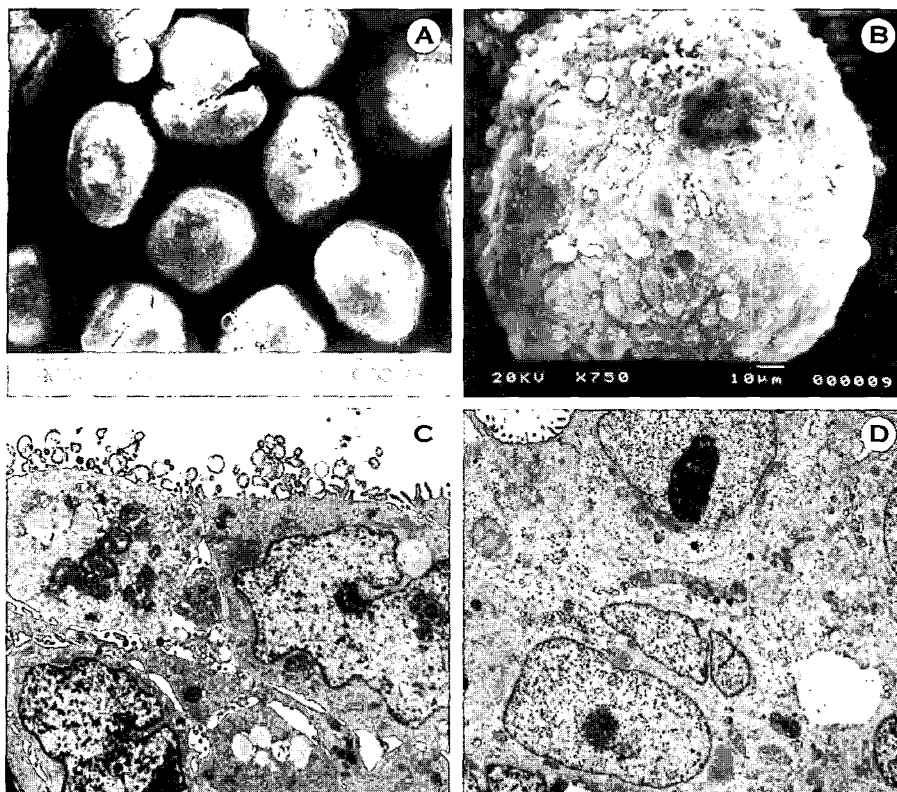


Fig. 1. Scanning electron micrograph **(A, B)** and Transmission electron micrograph **(C, D)** of HT-29 spheroid cells. A (×75), B (×750), C (×6,000), D (×6,000).

spheroid cells. As a result of observing the acquired cells under scanning electron microscope, it was found that similarly to the shape of *in vivo* cancer, an approx. 1 mm-dia spheroid was formed. In addition to that, it was shown that the spheroid consists of smaller spheroids - like sphere - where plenty of monolayer cells are accumulated. Each smaller spheroid cells were generally similar in the aspect of their size and shape. And, similarly to junctions between cells in the tissue, intercellular junctions on the surface of the spheroid was also seen (Fig. 1 A, B). Additionally, as a result of observation under transmission electron microscope, it was identified that morphologically diverse cells were distributed on the center of and around the spheroid cell owing to their different degrees of growth differentiation, which results in similar structure to the tissue. Intracellular structures showed normally well-developed forms. At the same time, as a result of observing the state of the nuclei, it could be considered that the cells had been normally differentiated well. Junctions between cells were also normally formed. Therefore, the overall shape exhibited the similar features to those of the tissue (Fig. 1 C, D).

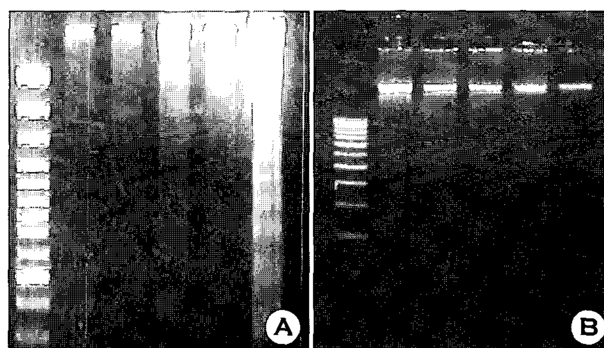


Fig. 2. Induction of DNA fragmentation in HT-29 monolayer cells **(A)** and HT-29 spheroid cells **(B)** treated with arsenic trioxide for 48 h. Lane M, DNA marker; lane 1, control; lane 2, 5 μM As_2O_3; lane 3, 10 μM As_2O_3; lane 4, 20 μM As_2O_3; lane 5, 30 μM As_2O_3.

2. Induction of apoptosis by arsenic trioxide and taxol

So as to make a comparison with the findings in HT-29 monolayer cell regarding whether arsenic trioxide and taxol induce apoptosis of HT-29 spheroid cell, the cell was treated with 5 μM, 10 μM, 20 μM and 30 μM of arsenic trioxide and with 50 nM, 100 nM, 200 nM and 400 nM of taxol. For combined administration, it was treated with 20 μM of arsenic trioxide plus 50 nM, 100 nM, 200 nM and 400 nM of taxol. 48 hours after the treatment, DNA was isolated,

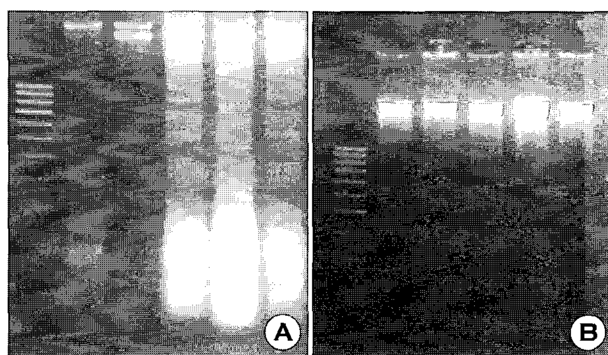


Fig. 3. Induction of DNA fragmentation in HT-29 monolayer cells **(A)** and HT-29 spheroid cells **(B)** treated with taxol for 48 h. Lane M, DNA marker; lane 1, control; lane 2, 50 nM taxol; lane 3, 100 nM taxol; lane 4, 200 nM taxol; lane 5, 400 nM taxol.

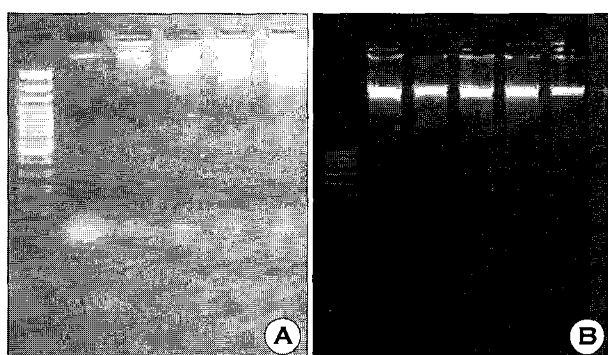


Fig. 4. Induction of DNA fragmentation in HT-29 monolayer cells **(A)** and HT-29 spheroid cells **(B)** treated with the combination of arsenic trioxide and taxol for 48 h. Lane M, DNA marker; lane 1, control; lane 2, 20 μM As_2O_3 and 50 nM taxol, lane 3, 20 μM As_2O_3 and 100 nM taxol; lane 4, 20 μM As_2O_3 and 200 nM taxol; lane 5, 20 μM As_2O_3 and 400 nM taxol.

1.8% agarose gel electrophoresis was performed and DNA fragmentation was identified. While DNA fragments were found at the level of 10 μM or higher in the monolayer cell treated with arsenic trioxide, they were not seen even at 30 μM, the highest level of the treatment group in the spheroid cell (Fig. 2). And, while the fragments were seen at 100 nM or higher in the monolayer cell treated with taxol, slight DNA fragmentation was seen in the spheroid cell (Fig. 3). At last, for combined treatment with both the drugs, the considerably decreased DNA fragmentation was identified in the monolayer cell compared with that treated with taxol only, whereas no DNA fragmentation was observed in the spheroid cell (Fig. 4).

3. Cytotoxic effects of arsenic trioxide and taxol

Before determining cytotoxicity of HT-29 spheroid cell,

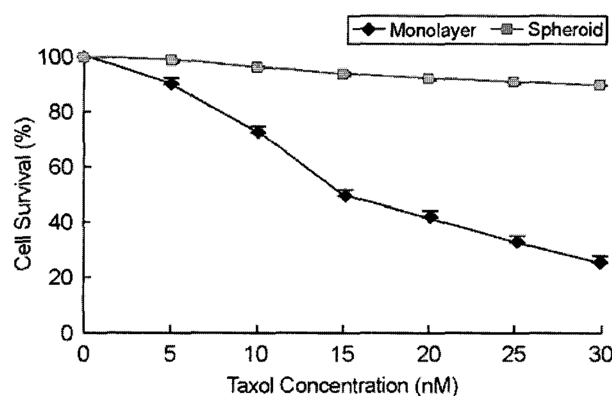


Fig. 5. Cytotoxic effect of taxol on the monolayer and the spheroid of HT-29 cells. The cells were incubated with indicated concentration of taxol for 48 h.

the relation between known cell count and measured optical density was examined so as to check that it reflects correctly the stability and the survival cell count of cytotoxicity test using premix solution. Identified by tryphan blue exclusion technique, survival cells were divided and put in a 96-well microplate at the level of 1.25×10^4, 2.5×10^4, 5×10^4, 1×10^5, 2×10^5 and 4×10^5 cells/well, and subsequently, treated with premix solution to measure their ODs (absorbance). As a result of drawing a standard curve between the measured ODs and cell count, it was shown that a directly proportional relation exists within the measured range, suggesting the survival cell count was reflected well. Based on these findings, the spheroid cell formed in the 96-well microplate where 1×10^4 cells were divided and put was treated with arsenic trioxide and taxol, separately and in the combined form at the same levels as in culturing the monolayer cell. After 48 hours, treatment with premix solution was performed, their ODs were measured at 490 nm and the measurements were converted into the unit of % survival. As the result, for taxol, 18 nM of IC_{50} value appeared in the monolayer cell, whereas resistance to the drug increased significantly in the spheroid cell, compared with the monolayer cell (Fig. 5). Meanwhile, for arsenic trioxide, 33 μM of IC_{50} value was indicated in the monolayer cell, whereas similarly to taxol, resistance to the drug increased significantly in the spheroid cell, compared with the monolayer cell (Fig. 6). For combined treatment, while antagonic effect was seen in the monolayer cell, there was no notable difference in the spheroid cell, from the experiment group treated with taxol only (Fig. 7).

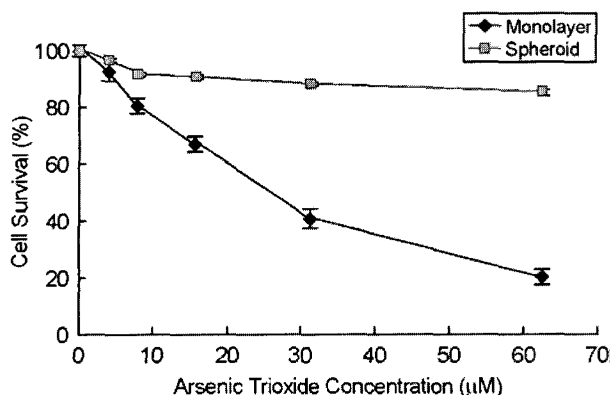
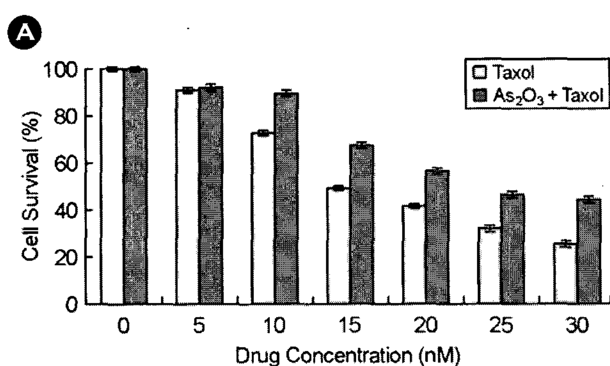


Fig. 6. Cytotoxic effect of arsenic trioxide on the monolayer and spheroid of HT-29 cells. The cells were incubated with indicated concentration of arsenic trioxide for 48 h.



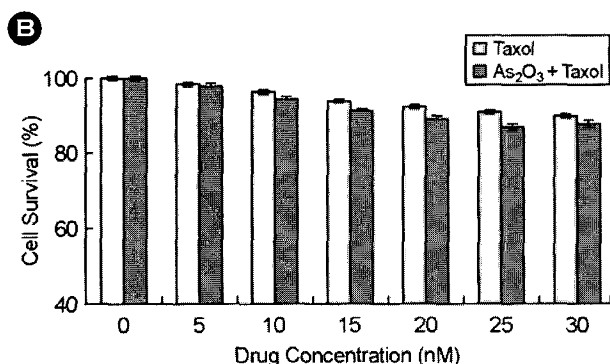


Fig. 7. Cytotoxic effect of 20 μM arseinc trioxide with various concentration of taxol in HT-29 monolayer cells **(A)** and the spheroid cells **(B)**. The cells were incubated with indicated concentration of taxol for 48 h.

4. Identification of drug penetration

As a result of freezing and sectioning the spheroid cell cultured in a 48-well microplate, applying DAPI staining to the resulting sections and observing them under fluorescence microscope, fluorescence was seen only on the surface, not inside the spheroid cell (Fig. 8). This finding suggests that DAPI molecules didn't penetrate into the spheroid cell.

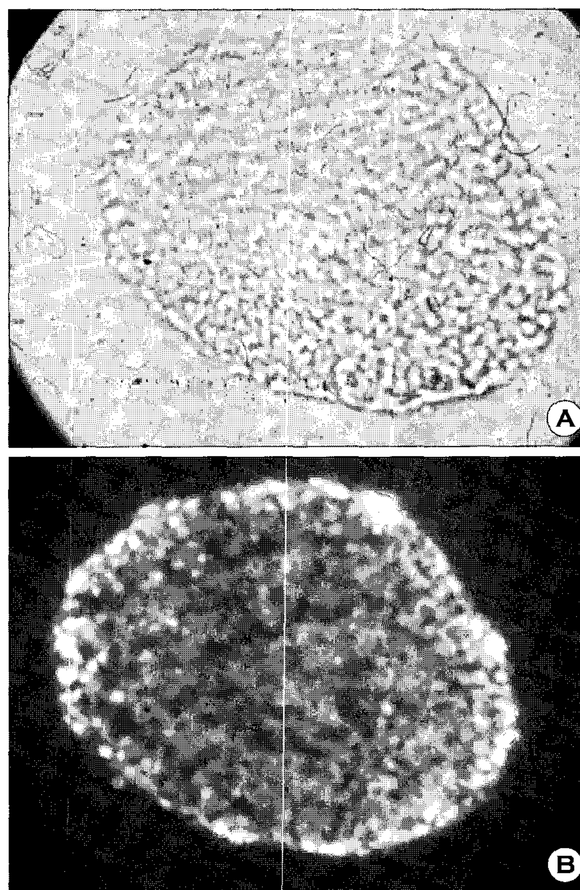


Fig. 8. DAPI staining of HT-29 spheroid cells. The spheroid cells were fixed and stained with DAPI and viewed by light **(A)** and fluorescence **(B)** microscopy under 400 × magnification.

DISCUSSION

Recently, there are many ongoing studies which expect additional effects or synergy by combining several kinds of anticancer agents with different targets so that more effective anticancer effects are achieved on the cancers resistant to anticancer agents, and studies on the therapeutic effects of combined drugs having similar mechanisms of action (Manon et al., 2002). Thus, this study was intended to investigate the effects of combined taxol and arsenic trioxide - both of them are well-known effective anticancer agents against HT-29 cell line, a cell line of colorectal cancer steadily increasing recently in Korea. As known generally, each of the agents showed cytotoxic effects in HT-29 human colorectal cancer cell, however combined administration of them led to relatively reduced cytotoxic effects.

Recent studies on the *in vitro* anticancer effects with the

sixty cell line panels customized for the types of diseases by the NCI reported that the results of experiments on *in vivo* solid cancer maintained homeostasis. For the reason, in this study, spheroid cell with three-dimensional characteristics was acquired by means of multicellular spheroid culture technique. As a result of observing the cultured spheroid cell, the formed spheroid cell was a spheroid with 1 mm-diameter size, composed of smaller spheroid cells inside. It was identified that the biggest spheroid cells consisted of numerous monolayer cells. In addition, as a result of observing the inside of the spheroid cell under TEM, it was shown that the structure of the inside resembled that of the general tissue. On the basis of these findings, it was found that there is the possibility for the spheroid cell to be used as an experimental model for study on the effects of anticancer agents on solid cancers.

As a result of checking whether taxol and arsenic trioxide induce apoptosis, it was found that they didn't induce apoptosis in the spheroid cell even at the level where they induced severely apoptosis in the monolayer cell. Also for the result of drug cytotoxicity test, it was shown that more than 80% of the spheroid cell survived even when the IC_{50} values in the monolayer cell, that is, 18 nM or higher of taxol and 33 μM or higher of arsenic trioxide were applied. In other words, it was revealed that in the case of HT-29 human colorectal cancer cell, its spheroid cell was much more resistant to the two agents, compared with its monolayer cell. A study reported that this high resistance is due to that the agents are difficult to penetrate into a solid cancer (Nicholson et al., 1997; Desoize et al., 2000). In this connection, in this study, fluorescence microscopy was carried out using smaller DAPI molecules than those of the agents in order to investigate whether they penetrate into the spheroid cell. As the result, fluorescence was seen only on the surface of, not in the cell. This result suggests that the agents whose molecules are larger than DAPI molecules are never easy to penetrate into the spheroid cell. Furthermore, it tells why the spheroid cell is more resistant to the agents compared to the monolayer cell.

As shown in the above-mentioned results, there are many differences in the findings of the same experiments between the monolayer cell and the spheroid cell of HT-29 human colorectal cancer - the latter resembles a solid cancer more similarly compared with the former. In other words, it was found that it is unreasonable to apply the results with the monolayer cell under *in vitro* conditions, directly to solid cancer under *in vivo* conditions. Accordingly, it may be given as a conclusion that an experimental model closer to *in vivo* solid cancer is required to research the effects of anticancer agents on solid cancer. At the same time, the possibility to use the spheroid cell as such an experimental model was identified. In addition, further studies are to be performed in the future so as to ascertain the presumption, derived from the finding in fluorescence microscopy with DAPI molecules. The increased resistance of the agents results from that they are not easy to penetrate into solid cancer.

Acknowledgements

This work was supported in part by Yonsei University Research Fund of 2004 and a grant (2004) from the Maeji Academic Institute.

REFERENCES

Bhalla K, Ilerado AM, Tourkina E, Tang C, Mahoney ME, Huang Y. Taxol induces internucleosomal DNA fragmentation associated with programmed cell death in human myeloid leukemia cells. Leukemia 1993. 7: 563-568.

Carles G, Braguer D, Sabeur G, Briand C. The effect of combining antitubulin agents on differentiated and undifferentiated human colon cancer cells. Anticancer Drugs 1998. 9: 209-221.

Chiung-Tong C, Jessie LSA, Gan Y, Wientjes MG. Differential time dependency of antiproliferative and apoptotic effects of taxol in human prostate tumors. Urol Oncol. 1997. 3: 11-17.

Derry WB, Wilson L, Jordan MA. Stoichiometric binding of taxol suppresses microtubule dynamics. Biochemistry 1995. 34: 2203-2211.

Desoize B, Jean-Claude J. Multicellular resistance: a paradigm for clinical resistance? Crit Rev Oncol Hematol. 2000. 36: 193 -207.

Fracasso PM, Brady MF, Moore DH, Walker JL, Rose PG, Letvake L, Grogan TM, McGuire WP. Phase II study of paclitaxel and valspodar (psc 833) in refractory ovarian carcinoma: a gynecologic oncology group study. J Clin Oncol. 2001. 19: 2975-2982.

Kobayashi H, Shan M, Charles HG, Sean JK, Beverly AT, Robert SK. Acquired multicellulared-mediated resistance to alkylating agents in cancer. Proc Natl Acad Sci USA. 1993. 90: 3294 -3298.

Huang SC, Huang CY, Lee TC. Induction of mitosis-mediated apoptosis by sodium arsenite in HeLa S3 cells. Biochem Pharmacol. 2000. 60: 771-780.

Jiang S. Encyclopedia of chinese medecine. Shangia. 1986. pp 1620-1622. Scientific Publishing House. China.

Jordan MA, Toso RJ, Thrower D, Wilson L. Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations. Proc Natl Acad Sci USA. 1993. 90: 9552-9556.

Kohn EC, Reed E, Sarosy GA, Minasian L, Bauer KS, Bostick Bruton F, Kulpa V, Fuse E, Tompkins A, Noone M, Goldspiel B, Pluda J, Figg WD, Liotta LAA. Phase I trial of carboxylamido triazole and paclitaxel for relapsed solid tumors: potential efficacy of the combination and demonstration of pharmacokinetic interaction. Clin Cancer Res. 2001. 7: 1600-1609.

Long MH, Fairchild CR. Paclitaxel inhibits progression of mitotic cells to G1 phase by interference with spindle formation without affecting other microtubule functions during anaphase and telophase. Cancer Res. 1994. 54: 4355-4361.

Lopes NM, Adams EG, Pitts TW, Bhuyan BK. Cell kill kinetics and cell cycle effects of taxol on human and hamster ovarian cell line. Cancer Chemother Phromacol. 1993. 32: 235-242.

Manon C, Gerard C, Nicolas A, Soazig D, Joseph C, Claudette B, Diane B. Involvement of microtules and mitochondria in the antagonism of arsenic trioxide on paclitaxel-induced apoptosis. Biochem Pharmacol. 2002. 63: 1831-1842.

Maria Wartenberg FC, Ling M, Schallenberg AT, Baumer, Petrat K. Down-regulation of intrinsic P-glycoprotein expression in multicellular prostate tumor spheroids by reactive oxygen. J Biol Chem. 2001. 276: 17420-17428.

Milas L, Hunter NR, Kurdoglu B, Mason KA, Meyn R, Stephene LC, Peter LJ. Kinetics of mitotic arrest and apoptosis in murine mammary and ovarian tumors treated with taxol. Cancer Chemother Pharmacol. 1995. 35: 297-303.

Nabholtz JM, Slamon D. New adjuvant strategies for breast cancer: meeting the challenger of integrating chemotherapy and trastuzumab (Herceptin). Semin Oncol. 2001. 28: 1-12.

Nakagawa Y, Akao Y, Morikawa H, Katsu K, Naoe T, Ohishi N, Yagi K. Arsenic trioxide-induced apoptosis through oxidative stress in cells of colon cancer cell lines. Life Sci. 2002. 70: 2253-2269.

Nicholson KM, Bibby MC, Phillips RM. Influence of drug exposure parameters on the activity of paclitaxel in multicellular spheroids. Eur J Cancer. 1997. 33: 1291-1298.

Ratnaike RN. Acute and chronic arsenic toxicity. Postgrad Med J. 2003. 79: 391-396.

Rieder C, Schuitz A, Cote R, Sluder G. Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitor sister kinetochore attachment to the spindle. J Cell Biol. 1994. 127: 1304-1310.

Rowinsky EK, Donehower RC. Paclitaxel (Taxol). N Engl J Med. 1995. 332: 1004-1014.

Shen ZX, Chen GQ, Ni JH, Li XS, Xiong SM, Qiu QY, Zhu J, Tang W, Sun GL, Yang KQ, Chen Y, Zhou L, Fang ZW, Wang YT, Ma J, Zang P, Zhang TD, Chen SJ, Chen Z, Wang ZY. Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocic leukemia (APL): II Clinical efficacy and pharmacokinetics in relapsed patients. Blood 1997. 89: 3354-3360.

Soignet SL, Maslak P, Wang ZG, Jhanwar S, Warell RP. Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. N Engl J Med. 1998. 339: 1341-1348.

Sonoda T, H, Kobayashi H, Kaku T, Hirakawa T, Nakano H. Expression of angiogenesis factors in monolayer culture, multicellular spheroid and in vivo transplanted tumor by human ovarian cancer cell lines. Cancer Lett. 2003. 196: 229-237.

Gebel T. Confounding variables in the environmental toxicology of arsenic. Toxicology 2000. 144: 155-162.

Hirano Y, Ushiyama T, Suzuki K, Fujita K. Clinical application of in vitro chemosensitivity test with the histoculture drug response assay for urological cancers: wide distribution of inhibition rates in bladder cancer and renal cell cancer. Urol Res. 1999. 27: 483-488.

Hirano Y, Takayama T, Kageyama S, Ushiyama T, Suzuki K, Fujita K. Thymidine Phosphorylase and Dihydropyrimidine Dehydrogenase in Renal Cell Carcinoma: Relationship between Histological Parameters and Chemosensitivity to 5-Fluorouracil. Eur Urol. 2003. 43: 45-52.