Effect of 3′-azido-3′-deoxythymidine (AZT) on telomerase activity and proliferation of HO-8910 cell line of ovarian cancer

Hongmei Li*, Tianbao Songa, Weizhong Xub, Yuecheng Yub, Xiaoyan Xing, Du Huih

aKey Laboratory of Environment and Genes Related to Diseases of Education Ministry, School of Medicine, Xi’an Jiaotong University, Xi’an, 710061, China
bDepartment of Obstetrics and Gynecology, Xijing Hospital, Fourth Military Medical University, Xi’an 710032, China

[Editorial] Though the article of “Effect of 3′-azido-3′-deoxythymidine (AZT) on telomerase activity and proliferation of HO-8910 cell line of ovarian cancer” raises more questions than it answers, it presents an interesting new line of research that has potential for identifying an important drug target for the treatment of cancer.

Actively dividing cells, e.g. cancer cells, need to replicate the ends of their telomeres. The polynucleotide polymerase that performs this task, Telomerase, is critical to this function, and thus presents a rational target for anti-cancer therapeutics. Nucleoside analogs have been used to target other polynucleotide polymerases, e.g. retroviral reverse transcriptases. Thus, it is reasonable to conjecture that nucleoside analogs might be applied to this problem as well. There is an emerging literature examining the effects of AZT on cancer cell lines. Though it is clear that this drug can kill cancer cells, the dosages used in these studies are well above any biologically meaningful range, and is not clear that the cytotoxic effects are due to direct effects on telomerase activity, or simple general metabolic poisoning of cells. While this emerging field of inquiry may hold some promise, direct biochemical studies comparing rates of incorporation of nucleoside analogs into DNA by a variety of human polynucleotide polymerases (e.g. the various cellular DNA polymerases, RNA polymerases, and telomerase) needs to be investigated. Should a preferred set of substrates be identified for telomerase, this may very well become the foundation for a new class of anti-neoplastic therapies.

Abstract
Objective: To study the effect of 3′-azido-3′-deoxythymidine (AZT) on telomerase activity and the proliferation of ovarian cancer cells in vitro.

Methods: Telomerase activity was detected by enzyme linked immunosorbent assay (ELISA) in treated and untreated HO-8910 cells by AZT. The detection of cell viability was performed with 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyl tetrazolium bromide (MTT) assay and the ultrastructure of the cells was observed by electron microscopy. The apoptotic rate of the cells was measured by flow cytometry.

Results: AZT significantly inhibited telomerase activity of HO-8910 cells, and the effect was both time- and dose-dependent. The HO-8910 cells treated at different concentrations of AZT showed a significant reduction of cell viability and morphological changes of apoptosis. The apoptotic rate of the cells was measured by flow cytometry.

Conclusion: AZT could effectively inhibit both telomerase activity and proliferation of human ovarian cancer HO-8910 cells in vitro, suggesting that AZT may be used in the clinic treatment of ovarian cancer.

Keywords: 3′-azido-3′-deoxythymidine (AZT); Reverse transcriptase inhibitor; Telomerase; Proliferation; Ovarian carcinoma cell

*Corresponding author: Tel: 86 - 29 - 88069258; Fax: 86 - 29 - 88390885; E - mail: ylhmqq@yahoo.com.cn
Effect of ATZ on ovarian cancer cell

Introduction

Telomerase is a specialized reverse transcriptase (ribonucleoprotein polymerase) consisting of telomerase RNA and protein. Telomerase RNA with a short template element directs the synthesis of telomeric repeats at chromosome ends, maintains chromosomal stability, stabilizes telomere length, and leads to neoplasm occurrence and cell immortality.\textsuperscript{1,2} The telomerase activity was expressed in 85\% of human cancers but not or seldom in normal somatic cells and benign tumors.\textsuperscript{3,4} Telomerase activation was thought to be an essential event for tumor proliferation.\textsuperscript{5,6} 3′-azido-3′-deoxythymidine (AZT) belongs to nucleoside analogs, which is currently used in the treatment of acquired immunodeficiency syndrome (AIDS). The main effect of AZT is to inhibit the activity of reverse transcriptase and synthesis of virus.\textsuperscript{7} In recent years, researchers have found that AZT can inhibit many enzyme activities of cells in vitro, especially the telomerase activity. AZT can also inhibit reverse transcription process, activity of reverse transcriptase, telomerase activity and telomere expanding. Cell divisions and telomere shortening can also be inhibited by AZT. Thus cell stability is destructed, and cell proliferation and growth are inhibited.\textsuperscript{9,10}

Ovarian cancer is the most common malignant tumor of women, with high mortality and poor prognosis. Although advances in ovarian cancer treatment with cytoreductive surgery and chemotherapy have improved the survival rate in the last decade, prognosis remains poor in patients with ovarian carcinoma because of ineffective diagnosis and treatment. Therefore, it will be essential to explore novel forms of diagnosis and treatment. Numerous studies have demonstrated that telomerase is highly expressed in ovarian cancer.\textsuperscript{11,12,13} In this study, the inhibitory effect of AZT on telomerase activity and proliferation of ovarian cancer cells in vitro were examined and the possibility of AZT for clinical therapy of ovarian cancer was explored.

Materials and Methods

Regents

AZT was purchased from Sigma (St. Louis, MO, USA), telomerase polymerase chain reaction-enzyme linked immunosorbent assay (PCR-ELISA) Kit from Boehringer Manheim (Germany), RPMI-1640 from Hykolong Company (USA), and trypsin from DIFCO Company (USA). Penicillin and streptomycin were the products of Pharmaceutical Factory of Haerbin, (China), fetal calf serum was obtained from Zhejiang Evergreen Company (China), 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyl tetrazolium bromide (MTT) from Huamei Biological Company (China), and dimethyl sulfoxide from Jinshan Chemical Plant (Shanghai, China).

Cell line

Ovarian cancer cell line HO-8910 was developed in the laboratory of the Department of Obstetrics and Gynecology, Xijing Hospital, China. HO-8910 cells were cultured in PRMI 1640 supplemented with 100mg/ml streptomycin, 100mg/ml penicillin and 10\% fetal calf serum. The cells were incubated at 37\textdegree C in the presence of 5\% CO\textsubscript{2}. The cells at the phase of logarithmic growth were used for later experiments.

Telomerase activity assay

HO-8910 cells were randomly divided into the experimental group and the control group. The cells in the experimental group were kept in the culture medium with AZT at the concentrations of 0.5 mM, 0.8 mM, 1.0 mM and 1.5 mM for 24h, 48h and 72h, respectively. The same culture medium without AZT was used for the cells in the control group. The cells were collected at different time points by centrifugation at 2000g for 10 min at 4\textdegree C. The pelleted cells were resuspended in 200 \mu L lysis reagent and incubated on ice for 30 min followed by centrifugation at 12,000g for 25 min at 4\textdegree C.
In the control group, HO-8910 cells were treated for 10 min at 65°C. The telomerase activity of HO-8910 cells was measured using a telomerase PCR - ELISA Kit.

**MTT assay on suppression of tumor cell growth**

HO-8910 cells were cultured in PRMI 1640 containing 10% fetal calf serum, in which the cultured cells reached over 90% confluence in 96 well plates. Different concentrations of AZT (0.05 mM, 0.1 mM, 0.2 mM, 0.5 mM, 0.8 mM, 1.0 mM, 1.2 mM, 1.25 mM, 1.6 mM, 2.0 mM) were added to the cells in the experimental group while equal volumes of culture medium were added to the cells in the control group. After 24, 48 and 72h, 20 μL of MTT (5mg/mL) was added to each well and the cells were incubated for another 4h at 37°C. The supernatant was carefully removed, and 150 μL of dimethyl sulfoxide was added into each well and shaken for 10 min. Absorbance (A) of the samples was measured at 490 nm by an enzyme linked immunoassay meter. The inhibitory rate of tumor cells = (1 - A_{AZT} / A_{control}) ×100%.

**Ultrastructure of tumor cells**

HO-8910 cells were cultured in 100 mL flasks in PRMI 1640 containing 10% fetal calf serum. When the cells reached over 90% confluence, AZT was added to the cells in the experimental group at the final concentration of 0.8 mM or 1.2 mM, and equal volume of culture medium was added to the cells in the control group. Then all the cells in both groups were incubated for another 72h. After the incubation the cells were transferred to a fresh tube and collected by centrifugation at 2000g for 10 min. 2 mL of 20g/L glutaraldehyde was then added and the cells were fixed for 2h. After the cells were embedded and ultrathin sections were cut, the ultrastructure of the cells were observed by transmission electron microscope.

**Cell cycle definition**

HO-8910 cells (5×10^5) in the experimental group were added to culture medium containing 0.8 mM or 1.2 mM AZT and the cells in the control group were added to culture medium without AZT. After incubation for 72h, the cells in both groups were collected by centrifugation at 1000g for 10 min and the pellet was carefully washed twice with 0.01M phosphate buffered saline (pH 7.3). Then 1mL of 70% ethanol was added to the collected cells and the cells were kept at 4°C for further processing. After washed twice with 0.01M phosphate buffered saline, the cells were stained with 300 μL of propidium iodide for 30 min. The cell cycle was detected by flow cytometer.

**Statistical analysis**

The SPSS 12.0 software was used to determine the statistical significance of the differences between the two groups. The result was examined by the Repeated Measure ANOVA. P value less than 0.05 was considered significant.

**Results**

**Inhibition of telomerase activity of HO-8910 cells by AZT**

Telomerase activity of HO-8910 cells was inhibited by AZT at 0.5 mM for 24h. The inhibitory effect was significantly increased with the elevation of drug concentration and elongation of exposure time. (P<0.05, Table 1).

**Suppression of cell growth with MTT assay**

The inhibition on HO-8910 cell growth of AZT at different concentrations for 24h, 48h and 72h, respectively, was detected with MTT assay. The results showed that lower concentrations of AZT had a slight inhibitory effect on HO-8910 cells. With the increase of AZT concentration, the A value was decreased and the inhibitory rate was increased and the effect was dose
Effect of ATZ on ovarian cancer cell

- dependent. Similarly, with the elongation of acting time, the inhibitory rate was also increased in a time-dependent manner. The minimum inhibitory effect appeared with 0.8 mM at 24 h, with 0.5 mM AZT at 48 h, and with 0.1 mM AZT at 72 h (Fig. 1, Table 2).

Ultrastructural changes of HO-8910 cells

The cells in the control group were observed to be polygon- or anomalus-shaped with various sizes by electron microscope. There were lots of long and thin microvilli on cellular surface (Fig. 2A). These cells had well-developed organelles, including mitochondria, rough endoplasmic reticulum, lysosomes and free ribosomes, etc. The nucleus was large with many karyokinesis, and abnormal nucleus mitosis was observed. After treated with AZT at 0.8 mM for 72 h, some HO-8910 cells became swollen with dilated endoplasmic reticulum and fewer mitochondria appeared when compared with the cells in the control group (Fig. 2B). After treated with AZT at 1.2 mM for 72 h, the microvilli of the cells were decreased in number and plenty of bubble-shaped convex appeared on cell surface. The chromatin was condensed and aggregated against the nuclear membrane, the structure of which was intact (Fig. 2C).

Table 1 Inhibition of AZT on telomerase activity of HO-8910 cells

<table>
<thead>
<tr>
<th>AZT Concentration (mM)</th>
<th>24h</th>
<th>48h</th>
<th>72h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.63±0.5</td>
<td>0.58±0.5</td>
<td>0.56±0.7</td>
</tr>
<tr>
<td>0.5</td>
<td>0.49±0.7*</td>
<td>0.46±0.7**</td>
<td>0.39±0.6**</td>
</tr>
<tr>
<td>0.8</td>
<td>0.45±0.6*</td>
<td>0.40±0.8**</td>
<td>0.35±0.6**</td>
</tr>
<tr>
<td>1.0</td>
<td>0.34±0.6*</td>
<td>0.29±0.5**</td>
<td>0.22±0.4**</td>
</tr>
<tr>
<td>1.5</td>
<td>0.30±0.5*</td>
<td>0.28±0.2**</td>
<td>0.16±0.3**</td>
</tr>
</tbody>
</table>

*: P<0.05, vs. control group, #: P<0.05, vs. value at 24h.

Table 2 Inhibitory rate (%) of AZT on the growth of HO-8910 cells

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>AZT Concentrations (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>72</td>
<td>4</td>
</tr>
<tr>
<td>48</td>
<td>6</td>
</tr>
<tr>
<td>24</td>
<td>7</td>
</tr>
</tbody>
</table>

- Fig. 1 A value of HO-8910 cells exposed to AZT at different concentrations for 24h, 48h and 72h.
- Fig. 2 Morphologic changes of HO-8910 cells in the control group (A) and in the groups treated with AZT at 0.8 mM (B) and 1.2 mM (C) for 72h.
Changes of cell cycle

The cells treated with AZT at various concentrations had cell cycles different from those of the cells in the control group. After exposure to 0.8 mM AZT for 72h, the percentage of the cells in the phase G1 dropped from 57.6% (in control group) to 1.1%, the percentage of the phase G2/M cells rose from 15.5% to 57.6%, and the percentage of the phase S cells rose from 26.9% to 41.2%. When AZT was used at the concentration of 1.2 mM, the percentage of the phase G1 cells rose to 67.7%, the phase G2 cells fell to 12.2%, and the phase S cells dropped to 20.1%. The apoptosis cusp was observed and the apoptotic rate was 14.2% in the group of 1.2 mM AZT (Fig. 3).

Discussion

AZT is one of the nucleoside analogs and capable of cutting out reverse transcriptase chain by competing conjugation with RNA template and normal mono-ribo-nucleotide. AZT inhibits the activity of reverse transcriptase and process of reverse transcription, thus finally inhibits cell growth. Therefore, it was named reverse transcriptase inhibitor. AZT was originally used to treat AIDS. AZT not only inhibits certain enzyme activities of cells but also induces apoptosis of lymphoma correlated with AIDS. It is reported that AZT can inhibit telomerase activity and proliferation of mammary cancer cells, cervix cancer cells, and other cancer cells in vitro.

In our study, we observed the effects of AZT at different concentrations on HO-8910 cells of ovarian cancer and their results at different time points. Our findings showed that AZT effectively inhibited telomerase activity and proliferation of human ovarian cancer HO-8910 cells in vitro, and that the viability of cancer cells had a significant reduction. At the same time points, the inhibitory effect of AZT was increased with the elevation of drug concentration; at the same drug concentration, its inhibitory effect was significantly increased with elongation of time. The inhibitory effect was both time- and dose-dependent. The notable cytotoxic effect of AZT on HO-8910 cells at 0.1 mM was observed 72h after drug treatment and at 0.8 mM 24h after AZT administration. The results indicated that drug concentration and exposure time of AZT were important for the suppressed growth of ovarian cancer HO-8910 cells. Cellular swelling, endoplasmic reticulum expanding and mitochondrion were observed to decrease in number by electron microscopy. The chromatin of cells was condensed and peripherally located. Flow cytometry detection showed that the cells treated with the drug at different concentrations had different cell cycles. The cells treated at low concentrations of AZT (0.8 mM) for 72h were gathered
Effect of ATZ on ovarian cancer cell

at the phase G₂/M. When the cells were treated with 1.2 mM of AZT for 72h the cells at the phase G₁ increased and the cells at the phase S decreased. The apoptotic peak was detected by flow cytometry and the apoptotic rate was 14.2%. The growth suppression of cells might be related to apoptosis. Nevertheless, it is still unknown whether AZT itself or apoptosis inhibiting genes induce the apoptosis, and the mechanism of cell apoptosis needs further investigation.

We also found that some cells survived at the 2.0 mM of AZT concentration due to their resistance to AZT. The development of these cells and their relation to drug resistance of tumor cells require further study.

Some researchers reported that the effect of tumor chemotherapy was more obvious when AZT was combined with drugs for chemotherapy. AZT may act as a synergist of drug for chemotherapy.¹⁹, ²⁰ In our previous research we studied the effect of AZT combined with the drugs of chemotherapy on the growth of human ovarian cancer line HO-8910. The inhibitory effect on cancer cells of AZT combined with adriamycin or carboplatin was stronger than that of each drug singly used.²¹

Chemotherapy is an essential therapeutic approach in the treatment of ovarian cancer. The results from our research showed that AZT had some significantly inhibitory effects on telomerase activity and proliferation of HO-8910 cell line of ovarian cancer cells. The ideal targeting strategy for tumor therapy should focus on some essential components present in tumor cells but not in normal cells. Telomerase is an essential condition of cell immortalization and an important factor of tumor development. Theoretically, the inhibition of telomerase activity by AZT may become a new treatment target because AZT can directly inhibit telomerase, which is absent in normal somatic cells. AZT can inhibit the growth of tumor cells with little injury to most normal cells by inhibiting telomerase activity. Moreover, it may also increase the specific effect and reduce the side effects of chemotherapy.

Since most tumor cells have telomerase activity, treating cancers by inhibiting telomerase activity is of great prospect.²² Therefore, the application of AZT might provide a novel approach to the targeted treatment of ovarian cancer and other cancers. However, the mechanism by which AZT inhibits the cell growth and interrupts the cell cycle of ovarian cancer needs further investigation.

References