Effect of combination of all-trans retinoic acid and arsenic trioxide on apoptosis of acute promyelocytic leukemia cells

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Abstract: To study the effect of all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) combination treatment on apoptosis of acute promyelocytic leukemia cells (NB4), inflammation and prognosis. The effect of ATRA-ATO combination on the proliferation of NB4 was determined using MTT assay. Apoptosis of NB4 cells was assessed with TUNEL assay. The effect of ATRA-As2O3 combination on the expressions of IL-6 and TNF-α in NB4 cells was determined using ELISA kits, while its effect on the quality of life of 25 acute promyelocytic leukemia patients admitted to our hospital was scored, as an index of prognosis. The combination treatment with ATRA and ATO significantly inhibited the proliferation of NB4 cells and promoted their apoptosis, relative to the model group. In addition, the combination treatment reduced serum IL-6 and TNF-α levels in patients with acute promyelocytic leukemia, and improve their quality of life and survival. Combination treatment with ATRA and ATO significantly inhibits the proliferation of NB4 cells and promotes their apoptosis, and reduces inflammatory responses in patients with acute promyelocytic leukemia, while improving their quality of life and prognosis.

Key words: Acute promyelocytic leukemia; All-trans retinoic acid; Arsenic trioxide; NB4 cells; Inflammation.

Introduction

Acute promyelocytic leukemia (APL) is a special type of acute myeloid leukemia which is characterized by rapid onset and progression, and it accounts for 10 – 15 % of leukemia cases, with majority of patients dying from complications before complete remission (CR) (1, 2). It belongs to the most lethal M3 subtype of acute leukemia, and over 90 % of patients with APL have characteristic cytogenetic changes. Patients with chromosome dislocation have retinoic acid receptor (PML-RARα) fusion gene. The characteristic features of the disease are unlimited proliferation of promyelocytes and inhibition of apoptosis (3, 4). In the past 20 years, a lot of progress has been made on studies on APL. The discovery of the therapeutic effects of ATRA and ATO has become a milestone in the treatment of APL (5). All-trans retinoic acid (ATRA), also known as retinoic acid (chemical formula = C20H28O2) is a metabolite of vitamin A in animals. In 1986, ATRA was used in the treatment of APL in China, with about 90 % complete remission. The complete remission of APL was increased to about 90 - 95 % with addition of anthracyclines, and the incidence of complications was significantly reduced (6). In the 1990s, ATO was used to treat APL, with significant therapeutic effects. Arsenic trioxide (ATO), one of the oldest poisons, is odorless and tasteless, and was named pishuang (arsenic) due to its white frost-like powder appearance (7, 8). With deepening of research, the treatment of APL has been continuously improved. The treatment has changed from the use of a single drug to the application of combination of two drugs, so as to promote the dual effect of apoptosis and differentiation of APL cells. The combination treatment plays an important role in maintaining complete remission and improved prognosis of APL (9). However, there are very limited studies on the effect of ATRA - ATO combination treatment on APL, and the effect of the combination treatment on the quality of life of the patients. The present study was carried out to investigate the effect of ATRA-ATO combination on proliferation and apoptosis of APL NB4 cells, and its effect on serum IL-6 and TNF-α levels in APL patients, as well as their quality of life and overall survival.

Materials and Methods

Reagents and Animals

Reagents

Fetal bovine serum (FBS), penicillin/streptomycin (P/S) and trypsin were purchased from American Invitrogen Company; PBS tablets and paraformaldehyde were products of Beijing Suo Laibao Technology Co., Ltd; MTT kits were purchased from Byuntian Institute of Biotechnology, while IL-6 and TNF-α ELISA kits were products of Nanjing Institute of Bioengineering. Culture flasks and plates were obtained from Corning, USA, while TUNEL assay kits were purchased from R&D Systems, USA. Arsenic trioxide (ATO) and ATRA were purchased from American Sigma Corporation.
Results

ATRA - ATO combination inhibited the proliferation of NB4 cells

The proliferation of NB4 cells was determined using MTT assay kit at 24, 48 and 72 h after treating with the combination of 1 μmol/L ATRA and 32 μmol/L ATO. The proliferation of NB4 cells was significantly inhibited at 48 h by the combination treatment, when compared with the control group ($p < 0.05$). The inhibition gradually and significantly increased with time ($p < 0.05$). These results are shown in Figure 1.

ATRA - ATO combination treatment enhanced apoptosis of NB4 cells

As shown in Figure 2, apoptosis in the combination treatment group was significantly increased, when compared with the control group ($p < 0.05$).

ATRA - ATO combination downregulated the expressions of IL-6 and TNF-α in APL patients

The levels of IL-6 and TNF-α in APL patients were
Table 1. Effect of ATRA - ATO combination on the levels of IL-6, TNF-α in acute promyelocytic leukemia patients.

<table>
<thead>
<tr>
<th>List</th>
<th>Control</th>
<th>ATRA/ATO</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/ml)</td>
<td>120.34 ± 10.46</td>
<td>75.84 ± 8.39*</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>154.89 ± 11.23</td>
<td>93.27 ± 11.52*</td>
</tr>
</tbody>
</table>

*p < 0.05 (ATRA - ATO treatment compared with control group).

Table 2. Effect of ATRA - ATO combination treatment on the quality of life of acute promyelocytic leukemia patients.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>ATRA/ATO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical function</td>
<td>54.38 ± 8.89</td>
<td>62.34 ± 7.53</td>
</tr>
<tr>
<td>Role function</td>
<td>29.47 ± 5.23</td>
<td>49.47 ± 5.04*</td>
</tr>
<tr>
<td>Emotional function</td>
<td>67.36 ± 7.12</td>
<td>83.87 ± 8.39*</td>
</tr>
<tr>
<td>Cognitive function</td>
<td>67.39 ± 8.35</td>
<td>89.46 ± 9.18*</td>
</tr>
<tr>
<td>Social function</td>
<td>40.74 ± 5.84</td>
<td>58.62 ± 6.72*</td>
</tr>
</tbody>
</table>

*p < 0.05 (ATRA - ATO combination treatment group compared with control group).

Table 3. Effect of ATRA-ATO combination treatment on prognosis of acute promyelocytic leukemia patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>ATRA/ATO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early mortality (%)</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Complete remission (%)</td>
<td>37</td>
<td>45*</td>
</tr>
<tr>
<td>Time taken to achieve complete remission (days)</td>
<td>38.5 ± 4.2</td>
<td>21.8 ± 4.6*</td>
</tr>
<tr>
<td>Recurrence (%)</td>
<td>17</td>
<td>6*</td>
</tr>
</tbody>
</table>

*p < 0.05 (ATRA-ATO combination treatment group vs. control group).

Discussion

Acute promyelocytic leukemia (APL) is an acute form of myeloid leukemia which is currently clinically treated using induction therapy, post-remission therapy, new therapy and bone marrow transplantation (13). It is pathologically characterized using immunology, cell morphology, molecular biology and chromosomology. In chromosomology, the pathological event is the mutual translocation of chromosomes 15 and 17. Indeed, 98% of the patients have fusion genes. The PML-RARα proteins form dimeric complexes and cause disseminated intravascular coagulation. A large number of granular promyelocytes are produced in the bone marrow, leading to coagulopathy and bleeding tendency (14, 15). Prior to the use of targeted drugs in clinics, the mortality rate of APL was extremely high. However, with deepening of research on APL, many new treatment methods have emerged (16, 17). Acute promyelocytic leukemia (APL) was a type of acute myeloid leukemia with the worst prognosis until ATRA and ATO were applied in clinical practice (16, 17). The combination therapy of ATRA and ATO has become the first-line treatment for patients who are intolerant of anthracycline (18).

In a previous study, Liu et al. labeled a GFP model to study the effect of ATRA or ATO administration on the differentiation of APL cells. It was found that APL cells differentiated significantly and PML-RARα protein degradation was enhanced after administration of the drug (19). The complete remission of patients was significantly increased after treatment with ATRA or ATO (20). In another study (21), it was found that HOXA7 gene decreased after ATRA treatment, thereby inhibiting the proliferation of NB4 cells. Further studies found (21) that ATRA also changed the morphology of NB4 cells. Thus, HOXA7 may be a candidate gene for treating APL, which is an improvement on the molecular basis for the treatment of APL.

In the present study, APL NB4 cells were first used significantly lower in the combination treatment group than that in the control group (p < 0.05). However, there was no significant difference in early mortality between the two groups.
to study the effects of ATRA- ATO combination on their proliferation and apoptosis. It was found that NB4 cell proliferation was inhibited after 48 h of ATRA and ATO treatment. Besides, the inhibition became more significant with time. The apoptosis of NB4 cells was detected with TUNEL assay. The apoptosis of NB4 cells significantly increased after ATRA and ATO combination treatment, suggesting that the combination treatment inhibited the proliferation of NB4 cells and promoted their apoptosis. This finding provides experimental basis for the use of these agents in clinical practice. In addition, it was found that the combination of ATRA and ATO significantly reduced the serum levels of IL-6 and TNF-α in patients, indicating that the combination treatment effectively controls patient's inflammatory response. After the combination treatment, the emotional, cognitive, and social functions of patients were significantly enhanced, indicating improved quality of life. The findings on the effect of the combined treatment on patients’ prognosis suggest that it enhances complete remission, shortens the time taken to achieve complete remission, reduces recurrence, and effectively improves prognosis.

The results of this study suggest that ATRA combined with ATO can significantly inhibit the proliferation and promote apoptosis of NB4 cells. Furthermore, the combination of ATRA and ATO significantly enhances the quality of life of patients, shortens the time taken to achieve complete remission, and decreases recurrence of APL. Thus, the combination therapy merits clinical application.

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None.

Interest conflict
There is no conflict of interest to be declared by the author.

Author’s contribution
We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors, all authors read and approved the manuscript for publication.

Qiyuan Sun conceived and designed the study, Jiane Hu, Qiyuan Sun, Wei Fang, Qinglin Wang collected and analysed the data, Jiane Hu wrote the manuscript.

References