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Tanshinone IIA inhibits acute promyelocytic leukemia cell proliferation and induces their apoptosis in vivo

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ABSTRACT

Tanshinone IIA (TanIIA) is a traditional Chinese agent and has been widely used for treatment of cardiovascular diseases. Our previous study has shown that TanIIA can induce the differentiation of acute promyelocytic leukemia (APL) cells by increasing C/EBPβexpression and induce APL cell apoptosis in vitro. In this study, we evaluated the activity of TanIIA against APL in vivo. We found that treatment with TanIIA prevented APL-mediated reduction in body weights. Treatment with TanIIA inhibited the proliferation of APL cells and triggered APL cell apoptosis and differentiation in vivo. Treatment with TanIIA significantly prolonged the survival of APL-bearing mice. Our data indicate that TanIIA has potent anti-APL activity with little adverse effect.

1. Introduction

Acute promyelocytic leukemia (APL) is characterized by a balanced reciprocal translocation between chromosomes 15 and 17, which results in the fusion between the promyelocytic leukemia (PML) gene and retinoic acid receptor α (RARa). Previously, APL had high mortality. Therapeutic strategy to induce the differentiation of APL cells by all trans retinoic acid (ATRA) and arsenic trioxide (ATO) has shifted APL from a highly fetal disease to a highly curable one [1]. Previous studies have shown that ATRA can induce APL cell terminal differentiation and ATO can induce APL cell apoptosis by rapid degradation of PML/PML-RARa. However, APL patients with ATRA and ATO treatment develop severe side effects and 48% of patients may develop the ATRA-related differentiation syndrome [2–6]. Furthermore, some patients with ATO treatment may display Q–Tc prolongation to >500 ms [7]. In addition, treatment with 7.5–10 μg/g ATO results in hepatic necrosis in animals [8]. Hence, the safety of long-term application of ATRA and ATO is concerned. Therefore, discovery of new safe and effective reagents to induce APL terminal differentiation will be of great significance.

Tanshinone IIA (TanIIA) is an extract of Danshen, a traditional Chinese medicine, which has been used for many years in the clinic. Previous studies have demonstrated that TanIIA has potent antioxidant and anti-inflammatory activities [9,10]. Importantly, TanIIA is a relatively safe reagent with few side effects [11,12]. Our previous study has shown that TanIIA can induce ATRA-sensitive APL cell differentiation by upregulating CEBPβexpression and trigger ATRA-resistant APL cell apoptosis in vitro [13]. In this study, we further evaluated the effect of TanIIA on APL in a human leukemia xenograft model.

2. Methods

2.1. Reagents

TanIIA was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). A TanIIA stock solution [50 mM in dimethyl sulfoxide (DMSO); Sigma, St. Louis, MO] was prepared and diluted to working concentration immediately before use. ATO solution (0.1%) for intraperitoneal administration was purchased from Harbin Yita Pharmaceutical (Heilongjiang, China).

2.2. Cell culture

NB4 cells with chromosomal translocation t(15;17) were originally isolated from long-term cultures of leukemia blast cells on bone-marrow stromal fibroblasts by Lanotte et al. [14], and were provided

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by Ruijing Hospital, Shanghai. NB4 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C in a saturated humidified incubator of 5% CO_2. The cells in logarithmic growth phase were used for further experiments.

2.3. Animals

All animal studies were approved by the Animal Care and Use Committee of Sichuan University. An APL mouse model was established, as described previously [15]. Briefly, female NOD/scid mice (4 weeks old) were obtained from the Medical Institute of Experimental Animals of Chinese Academy of Medical Sciences, Beijing and were housed in a specific pathogen-free (SPF) facility at the Lab Animal Center of Sichuan University. The mice were injected intraperitoneally (i.p) with NB4 cells ($1 \times l0^6$), randomized at 7 days later, and treated i.p with saline (control), 5 mg/kg ATO (ATO), 10 mg/kg TanIIA (10 mg TanIIA) or 100 mg/kg TanIIA (100 mg TanIIA) daily for 14 consecutive days ($n = 10$ per group). The mice were closely monitored for their body weights (every 5 days) up to 80 days post-inoculation and sacrificed if any signs of approaching death appeared.

2.4. Peripheral blood cells

Peripheral blood samples were collected from individual mice at days 7, 21, 28 and 35 post-inoculation and then smeared on glass-slides. The cells were stained with Wright's solution and examined under a microscope.

2.5. Histological and cytological analyses

At the end of the experiment, the liver, spleen, and lung of individual mice were dissected, fixed in 4% formaldehyde, and embedded in paraffin. The tissue sections at 5 μm were stained with hematoxylin/eosin or May–Grünwald–Giemsa and examined under a microscope. The frequency of apoptotic cells in the liver, spleen, and lung of individual mice was determined by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assays using the in situ cell death detection kit, according to the manufacturers' instruction (Boehringer Mannheim). The proliferation rates of cells in the liver, spleen, and lung of individual mice were determined by immunohistochemistry using anti-Ki67 staining. Briefly, the liver sections (5 μm) were treated with 3% H_2O_2 in methanol to block endogenous peroxidase activity, and subjected to antigen retrieval in a 0.1 M sodium citrate buffer. The sections were incubated with an anti-Ki67 monoclonal antibody (Santa Cruz Biotech, Santa Cruz, USA) at 4 °C overnight. After being washed, the bound antibodies were detected with HRP-conjugated second antibodies, and visualized with DAB solution. The percentages of proliferative or apoptotic cells in five high fields (magnification ×400) were counted in a blinded manner.

2.6. Differentiation and apoptosis analyses

Ascites cells were collected from mice at 30 days, smeared on glass-slides. The cells were stained with Wright's solution and examined under a microscope. The differentiation and apoptosis of ascites cells were determined by flow cytometry. Briefly, ascites cells (5×10^5/tube) were stained in duplicate with PE-anti-CD11b and the percentages of CD11b + differentiated APL cells were determined by flow cytometry on FACSAria ((Becton Dickinson, San Jose, USA). In addition, ascites cells (5×10^5/tube) were stained in duplicate with FITC-anti-Annexin-V and PI in the dark for 30 min at room temperature. The percentages of apoptotic cells were determined by flow cytometry.

2.7. Statistical analysis

Data are expressed as the mean ± 95% confidence interval (C.I.). The survival of different groups of mice was estimated by the Kaplan–Meier method and analyzed by the log-rank test. The difference in the percentage of CD11b +, TUNEL + and Ki67 + cells in the different groups of mice was analyzed by one-way ANOVA. Statistical analysis was performed by using the SPSS 16.0 software (SPSS, Chicago). A two-side P-value of <0.05 was considered statistically significant.

3. Results

3.1. TanIIA prolongs the survival of APL-bearing mice

To test the safety of TanIIA treatment, NOD/SCID mice were injected i.p with NB4 cells to induce APL and treated with or without, 10, 100 mg/kg TanIIA, or ATO beginning on day 7 for two weeks. Analysis of peripheral blood at 0 day indicated that APL cells with typical morphological characteristics were present in all mice (Fig. 1A). However, there was no significant difference in the frequency of circulating APL cells among the different groups of mice (data not shown). These indicated the establishment of APL model. Treatment with TanIIA and ATO significantly prolonged the survival of APL-bearing mice and the mean survival time of the control mice was 37 days, which was significantly shorter than 45 days, 48 days, and 51 days of the mice treated with 10 mg TanIIA, 100 mg TanIIA and ATO, respectively ($P > 0.05$, Fig. 1C). The mean survival time of the mice treated with 10 mg TanIIA seemed shorter than that of the mice with 100 mg TanIIA or ATO, but there is no statistically significant difference ($P > 0.05$).

It was notable that the some mice in the TanIIA and ATO treatment groups survived more than 80 days. Peripheral blood leukemic cells in these mice disappeared and WBC and platelet counts as well as hemoglobin level returned to normal. Furthermore, there was no detectable leukemia cells in the liver and spleen of mice. Together, these data indicated that treatment with TanIIA and ATO resulted in leukemia remission in mice.

3.2. TanIIA affects APL growth and is safe for mice

All mice with treatment had no tremor, deteriorated diet, and raised fur. The body weights of individual groups of mice were measured longitudinally (Fig. 2A). The body weights in the mice treated with either drug were significantly higher than that in the control at 30 days post-inoculation ($P < 0.05$).

There were 8 out of 10 control mice that developed ascites and peritoneal solid tumors while only 6 out of 10 mice in the 10 mg TanIIA group, 4 out of 10 in the 100 mg TanIIA group and 6 out of 10 in the ATO group developed tumor in enterocelia. The mean tumor size was 4.97 cm^3 (the control), 2.01 (TanIIA 10 mg), 1.08 (TanIIA 100 mg), and 1.85 cm^3 (ATO), respectively. Treatment with TanIIA or ATO significantly decreased tumor growth rate and reduced the tumor size by 40–70% ($P < 0.05$, Fig. 2B). It was notably that the time when we measured the size of tumor was the mice appeared any signs of approaching death in the control group was obviously shorter than any of the treatment groups, so that the growth time of tumor in the treatment groups was longer than that of the control group. We considered that the actual effect of TanIIA and ATO on APL was greater.

While ATO treatment induced some hepatocyte apoptosis, tissue edema and steatosis in the liver of mice, treatment with TanIIA at either dose did not cause any obviously morphological changes in the liver of mice. Collectively, these data indicated that treatment with TanIIA at the dose range was relatively safe in mice.