As4S4 Induced Apoptosis in HeLa Cells and Its Molecular Mechanism

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ABSTRACT

Objective: To investigate the As4S4 induced growth inhibition and apoptosis in HeLa cells and its possible relationship with cyclooxygenase-2 (COX-2).

Methods: HeLa cells were treated with various concentrations (7.5, 15, 30, 60 mg/L) of As4S4 at different times (12, 24, 36, 48, 60 h). Cell growth was measured by MTT. Apoptosis was detected by double staining flow cytometry (FCM). Levels of PGE2 were measured by radioimmunoassay. The expression of COX-2 protein was examined by Western blot analysis.

Results: After treated with different concentrations of As4S4, the growth of HeLa cells was suppressed significantly in a dose-and time-dependent manner. The IC50 of 24 h was 30 mg/L (P<0.01). As4S4 induced apoptosis with a apoptosis rates at 8.13%-62.36% by flow cytometry (FCM) in a dose-dependent manners. The release of PGE2 was reduced in HeLa cells with the values being (70.56±2.03), (48.58±2.28), (29.25±1.57) and (18.02±1.04) respectively, significantly different compared with control group (3.15±0.01) (P<0.01). As4S4 also inhibited the activity and expression of COX-2 in a dose dependent manner and down-regulated the expression of COX-2 protein greatly.

Conclusion: As4S4 could inhibit the proliferation and increase apoptosis in human HeLa cells. These effects may depend on the inhibition of the expression of COX-2 and PGE2 by As4S4.

Key words: As4S4; Cervical carcinoma; Apoptosis; Flow cytometry; Western blot

While arsenic has long been known as a poison and environmental carcinogen, its dramatic effect in the treatment of Acute Promyelocytic Leukemia (APL) has made its mechanism of action a topic of intense interest. Realgar is one kind of arsenics, and tetra-arsenic tetra-sulfide (As4S4) is a major component in realgar. In recent years, a series of studies have been undertaken in vitro and in vivo, and demonstrate that As4S4 is effective in the treatment of cancer such as Acute Promyelocytic Leukemia (APL)[1, 2]. But it is not reported in cervical cancer.

Cyclooxygenase-2 (COX-2) is a role rate-limiting enzyme involved in the conversion of arachidonic acid to prostaglandins, it has been identified to have a close relation with tumor genesis. There is abundant documented evidence of elevated expression of COX-2 in cervical tumors and a variety of other malignancies. The resultant high level Prostaglandin E(2) (PGE-2) production may play an important role in cell proliferation, modulation of apoptosis, angiogenesis, inflammation and immune surveillance[3, 4]. This study was designed to investigate the As4S4 induced growth inhibition and apoptosis in HeLa cells and its relationship with COX-2 and PGE-2.

MATERIALS AND METHODS

Materials

Human cervical cancer HeLa cell line was obtained from Cell Culture Center of Shanghai Science Academy. As4S4 and MTT were obtained from Sigma Co. Antibody against COX-2 (Mouse-anti-human monoclonal), PMSF, RPMI-1640, were purchased from Santa Cruz Biotechnology, Inc, USA. PGE-2-RIA was the product of Beijing Shangbo Biotechnology Company.
MTT Assay

HeLa cells were plated (1×10^5 per well) in 96-well plates and incubated for 24 h. Medium containing 0, 7.5, 15, 30, 60 mg/L of As4S4 was introduced into the wells of different experimental groups in triplicate. MTT was added to each well 12, 24, 36, 48, and 60 h later. After incubation at 37°C for 4 h, the MTT medium was removed and 100 μl of DMSO was added. Color reaction was measured by ELISA reader at the wavelength of 570 nm. Cell viability was assessed by the percent of absorbance of As4S4 treated cells to that of controls.

Flow Cytometry

Cells were incubated with 15, 30, 60 mg/L of As4S4 for 24 h and treated with 70% ethanol at 4°C for 24 h, then washed with PBS. After centrifugation, the supernatant was discarded. Cells were incubated in buffer solution at 37°C for 1 h, then 5 μl Annexin-V-FITC was added into 195 μl cell suspension, mixed well at room temperature for 10 min. After cells were washed by PBS for one time, cells were suspended again by 190 μl buffer solution, then 10 μl (20 μg) PI were added into tube. At last cells were detected by flow cytometry.

Radioimmunooassay

HeLa cells were plated (1×10^5 per well) in 96-well plates and incubated for 24 h. Experimental teams were given 7.5, 15, 30, 60 mg/L of As4S4 for 24 h. Supernatant was collected for PGE-2 detection.

Extraction of Total Protein and Western Blot

HeLa cells were treated with different concentrations of As4S4 (7.5-60 mg/L) for 24 h. After culture solution was discarded, the cells were washed and centrifuged. The solution (containing 0.1 mol/L NaCl, 0.01 mol/L Tris HCl, pH 7.6, 0.001 mol/L EDTA, 100 g/mL PMSF and 2 μg/mL Leupeptin) was used to lyse the cells for 30 min. After centrifugation at 7500 r/min for 10 min, the supernatant was collected. All above-mentioned operations were done under 4°C. Protein concentration was measured and samples with 25 μg protein were loaded and run on SDS polyacrylamide. Proteins were then transferred onto nitrocellulose membrane. The membrane was blocked with 5% fat-free milk for 1 h, then probed with antibodies against COX-2 and β-actin and kept at 4°C overnight. After being washed three times with PBS for 10 min, the membrane was incubated with horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG (1:2000) for 2 h. After washing, immunoreactive bands were visualized by ECL reagent. The results of X-ray film scanning were analyzed by using computer software. The experiments were performed more than three times. The mean was taken as the final result for analysis.

Statistical Method

Statistical analyses were carried out by using the statistic software SPSS12.0 and t-test.

RESULTS

Inhibitory Effect of As4S4 on HeLa Cells

The growth of the HeLa cells in the As4S4 group became slow to various degrees in a time- and dose-independent manner. After treatment with As4S4 (7.5-60 mg/L) for 12-60 h the inhibitory rates of HeLa cells ranged from 23.58% to 79.75% (P<0.01). The IC50 of 24 h was 30 mg/L (Tab. 1, Fig. 1).

Effects of As4S4 on Cell Apoptosis

When HeLa cells were exposed to As4S4 at 7.5-60 mg/L for 24 h, apoptosis rate increased with the rise of concentration. Compared with control group, apoptosis rates at 15-60 mg/L had significant difference (29.58±2.51%, 46.24±3.92%, 62.36±4.42% vs. 2.84±1.88%, P<0.01), while the apoptosis rate at 7.5 mg/L (8.13±1.13%) had no significance (Tab. 2).

Effect of As4S4 on the PGE-2 Level

After HeLa cells were treated with As4S4 at 7.5-60 mg/L for 24 h, PGE-2 levels in HeLa cells gradually decreased with the rise of concentration. Compared with control group, PGE-2 levels at 7.5-60 mg/L has significant difference (70.56±2.03,