Environmental Toxicology and Pharmacology 41 (2016) 127–131

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Synergistic effect of radon and sodium arsenite on DNA damage in HBE cells

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ARTICLE INFO

Article history:
Received 5 June 2015
Received in revised form
19 November 2015
Accepted 20 November 2015
Available online 2 December 2015

Keywords:
Synergistic effect
Radon
Sodium arsenite
DNA damage

ABSTRACT

Human epidemiological studies showed that radon and arsenic exposures are major risk factors for lung cancer in Yunnan tin miners. However, biological evidence for this phenomenon is absent. In this study, HBE cells were exposed to different concentrations of sodium arsenite, different radon exposure times, or a combination of these two factors. The results showed a synergistic effect of radon and sodium arsenite in cell cytotoxicity as determined by cell viability. Elevated intracellular ROS levels and increased DNA damage indexed by comet assay and γ-H2AX were detected. Moreover, DNA HR repair in terms of Rad51 declined when the cells were exposed to both radon and sodium arsenite. The synergistic effect of radon and sodium arsenite in HBE cells may be attributed to the enhanced DSBs and inhibited HR pathway upon co-exposure.

1. Introduction

The World Health Organization (WHO) reported about 12 million new cancer cases and 8 million cancer-related deaths annually in recent years. Approximately 19% of all cancers can be attributed to environmental and occupational factors, such as work settings. The International Agency for Research on Cancer has classified 29 chemical or physical agents as human carcinogens to which exposure is mainly occupational- and industrial processing-related (Boyle and Levin, 2008). For example, benzene in shoe production is associated with acute non-lymphocytic leukemia (Baan et al., 2009), and radiation in industrial and medical treatment is linked to breast and lung cancers. Exposure to arsenic in smelting non-ferrous metals and metallurgical industries is also connected with lung, skin, and urinary bladder cancers (Cheng et al., 2015; Muenyi et al., 2015; Recio-Vega et al., 2015).

High incidence of lung cancer has been reported among tin miners in Yunnan Province, China (Zhang and Yao, 1989), caused by simultaneous exposure to radon and arsenic in the working environment. A dose–response relationship exists between exposure to radon/arsenic and incidence of lung cancer in the miners (Sun and Yao, 2001; Yao and Fan, 2007). Previous epidemiological investigation concluded that the high incidence of lung cancer may be causally attributed to occupational exposure to radon combined with arsenic but primarily to radon (Sun and Yao, 2001).

We conducted a series of in vitro experiments by exposing human bronchial epidermal (HBE) cells to radon combined with sodium arsenite to verify the epidemiological conclusion obtained from the tin miner study. The results may help provide biological evidence of the two occupational carcinogens and mechanistic clues underlying their combined carcinogenic effects.

2. Materials and methods

2.1. Cell culture

The HBE cells were kindly provided by Professor Wen Chen of Zhongshan University. The cells were grown in DMEM (Wisent Corp., Canada) supplemented with 10% fetal bovine serum (FBS; Wisent Corp., Canada), penicillin (100 U/mL) (Wisent Corp., Canada), and streptomycin (100 μg/mL) (Wisent Corp., Canada) at 37 °C in 95% air and 5% CO_2 atmosphere.

2.2. Exposure of cell culture to arsenic and radon

Stock solution of 10 mM sodium arsenite (Merck KGaA, Germany) was prepared in doubled-distilled water and sterilized by passing through a 0.22 μm syringe filter. The working solutions were obtained by diluting the stock with DMEM containing 10% FBS at 0, 5, 50, 500, 5000, and 50,000 μmol/L. HBE cells were seeded onto a Transwell membrane (Corning, USA) at a density of

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http://dx.doi.org/10.1016/j.etap.2015.11.013
1382-6689/

1×10^5 cells 1 day before sodium arsenite exposure. The cells were then incubated with different concentrations of sodium arsenite for 24 h.

HBE cells were seeded onto the Transwell membrane with a 0.4 μm polyester membrane bottom at a density of 1×10^5 cells 1 day prior to radon exposure. The cells were placed in a gas chamber (Chinese Academy of Military Medical Sciences, Beijing, China) connected with a multifunctional radon chamber purchased from Donghua University in China. Radon and its progeny were produced by a radium source, and radon gas was pumped into the cell chamber using a Changhe pump machine (Model BT00-300, China) with a concentration of 20,000 Bq/m^3 for different time periods (0, 15, 30, 45, 60, and 75 min) to obtain different radiation dosages. After radon exposure, DMEM with 10% FBS was added to cells on the Transwell membrane and then cultured for 24 h.

Combined exposure to radon and sodium arsenite was performed according to the radon exposure method followed by slight modification. After radon exposure, DMEM with 10% FBS and different concentrations of sodium arsenite were added to the Transwell membrane.

2.3. Cytotoxicity test

HBE cells were trypsinized, resuspended in complete DMEM, and plated on 96-well plates at 1×10^4 cells/well for 1 day. Cell Counting Kit-8 (Dojindo, Japan) was used to determine the cytotoxicity in accordance with the manufacturer's instructions. The dose–effect curves of single or combined treatments were analyzed by the median effect method using Calcusyn Software (Biosoft, USA).

2.4. ROS measurement

HBE cells exposed to radon, sodium arsenite, and their combinations were digested by 0.25% trypsin and washed by PBS. A ROS Assay Kit (Beyotime, China) was used in accordance with the manufacturer's instructions.

2.5. Comet assay

Comet assay according to Jiang et al. (2012) was followed with minor modifications. The cells were lysed (pH 10) to unwind DNA in alkaline buffer (pH 13) for 1 h followed by electrophoresis for 30 min (20 V, 300 mA). After neutralization (pH 7.5), the cells were stained with ethidium bromide (20 mg/mL) on coded slides. A fluorescence microscope equipped with CASP software (CASP Lab, Poland) was applied to record the comet tail DNA (total DNA%), tail length (TL), and olive tail moment (OTM). A total of 50 comets were chosen and analyzed for each slide.

2.6. Western blot

The cells were lysed for 30 min on ice using extraction buffer [50 mM Tris–HCl, pH 8, 150 mM NaCl, 1% NP-40, 0.1% sodium dodecyl sulfate (SDS), and 1 mM phenylmethyl sulfonyl fluoride] and centrifuged at 13,000 rpm for 15 min to obtain the supernatants. The total protein extracts were measured by bicinchoninic acid (Thermo, USA). The protein samples were mixed with loading buffer, heated at 100 °C for 5 min, and subjected to SDS–polyacrylamide gel electrophoresis. The proteins were then transferred to PVDF (Millipore, USA) membranes under wet conditions. The anti-gamma H2A.X (phospho S139) antibody (Abcam, USA), anti-Rad51 antibody (Abcam, USA), and β-actin (Multisciences, China) were applied to probe the membranes for quantification. Secondary antibodies were conjugated to horseradish peroxidase. Signals were detected using the ECL system.

2.7. Statistical analysis

Results were obtained from three independent experiments, with each experiment performed in triplicate. Data were expressed as the mean ± SD. One-way ANOVA and Student's *t*-test with pairwise comparisons were used for statistical analysis. A P value < 0.05 indicated a significant difference between groups.

The median effect was calculated using the following equation:

$$\frac{f_a}{f_u} = \left(\frac{D}{D_m}\right)^m$$

$$\log\left(\frac{f_a}{f_u}\right) = m\,\log(D) - m\,\log(D_m)$$

where f_a represents the inhibition rate, f_u represents cellular viability, D represents dose, and m represents slope.

The combination index (CI) was calculated by the following equation:

$$CI = \frac{(D)_1}{(D_x)_1} + \frac{(D)_2}{(D_x)_2}$$

where $(Dx)_1$ and $(Dx)_2$ are the doses when x% of the inhibition rate is caused by one or two individual effects. $(D)_1$ and $(D)_2$ are the doses when x% of the inhibition rate is caused by the combination of one and two effects.

3. Results

3.1. Radon and sodium arsenite produce synergistic cytotoxicity on HBE cells

The survival curves of the HBE cells exposed to radon (20,000 Bq/m^3, 0–75 min) showed that cell viability declined as the exposure time was prolonged (Table 1). Cell viability also declined

Table 1
Changes in cell viability and synergistic effects after exposure to radon or/and sodium arsenite ($\bar{x} \pm$ S.D., $n = 3$).

Radon groups		Sodium arsenite		Combined exposure		
Exposure time (min)	Viability (%)	Exposure dose (μmol/L)	Viability (%)	Combined treatment	Viability (%)	CI
0	100	0	100	0	100	
15	99.96 ± 13.59	5	84.90 ± 13.76*	15 min + 5 μmol/L	52.95 ± 1.17*	0.24 ± 0.03
30	92.52 ± 7.32*	50	66.72 ± 8.18*	30 min + 50 μmol/L	41.59 ± 2.98*	0.38 ± 0.01
45	78.76 ± 7.15*	500	38.51 ± 8.44*	45 min + 500 μmol/L	27.49 ± 0.53*	0.43 ± 0.01
60	47.01 ± 3.83*	5000	12.44 ± 3.25*	60 min + 5000 μmol/L	10.81 ± 0.10*	0.36 ± 0.03
75	13.23 ± 0.20*	50000	2.47 ± 1.92*	75 min + 50,000 μmol/L	0.90 ± 0.77*	0.17 ± 0.11

The combination index (CI) were using the data of cell viability exposure to radon or/and sodium arsenite and calculated by the median-effect method of Chou and Talalay (Chou, 2010).

* Compared with control group, $P < 0.05$.