Mutat Res Gen Tox En 820 (2017) 19–25

Contents lists available at ScienceDirect

Mutat Res Gen Tox En

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

Adaptive response in mouse bone marrow stromal cells exposed to 900 MHz radiofrequency fields: Impact of poly (ADP-ribose) polymerase (PARP)

Qina He[1,a], Lin Zong[1,a], Yulong Sun[a], Vijayalaxmi[b], Thomas J. Prihoda[c], Jian Tong[a], Yi Cao[a,*]

[a] School of Public Health, Medical College of Soochow University, Suzhou Jiangsu Province, People's Republic of China
[b] Deparment of Radiology, University of Texas Health Science Center, San Antonio, TX, USA
[c] Deparment of Pathology, University of Texas Health Science Center, San Antonio, TX, USA

ARTICLE INFO

Keywords:
Mouse bone marrow stromal cells
Radiofrequency fields
Poly (ADP-ribose) polymerase-1
Adaptive response
DNA damage

ABSTRACT

This study examined whether non-ionizing radiofrequency fields (RF) exposure is capable of inducing poly (ADP-ribose) polymerase-1 (PARP-1) in bone marrow stromal cells (BMSCs) and whether it plays a role in RF-induced adaptive response (AR). Bone marrow stromal cells (BMSCs) were exposed to 900 MHz RF at 120 μW/cm^2 power flux density for 3 h/day for 5 days and then challenged with a genotoxic dose of 1.5 Gy gamma-radiation (GR). Some cells were also treated with 3-aminobenzamide (3-AB, 2 mM final concentration), a potent inhibitor of PARP-1. Un-exposed and sham (SH)-exposed control cells as well as positive control cells exposed to gamma radiation (GR) were included in the experiments. The expression of PARP-1 mRNA and its protein levels as well as single strand breaks in the DNA and the kinetics of their repair were evaluated at several times after exposures. The results indicated the following. (a) Cells exposed to RF alone showed significantly increased PARP-1 mRNA expression and its protein levels compared with those exposed to SH- and GR alone. (b) Treatment of RF-exposed cells with 3-AB had diminished such increase in PARP-1. (c) Cells exposed to RF + GR showed significantly decreased genetic damage as well as faster kinetics of repair compared with those exposed to GR alone. (d) Cells exposed to RF + 3-AB + GR showed no such decrease in genetic damage. Thus, the overall date suggested that non-ionizing RF exposure was capable of inducing PARP-1 which has a role in RF-induced AR.

1. Introduction

The phenomenon of adaptive response (AR) which was originally described in *Escherichia coli* [1] is well documented in scientific literature. Animal and human cells which were exposed *in vitro* and *in vivo* to extremely low and non-toxic doses of a genotoxic agent were reported to become resistant to the damage induced by subsequent exposure to a higher and toxic dose of the same or a similar genotoxic agent and, some underlying mechanisms were discussed [2]. In recent years, the existence of similar phenomenon has been reported in freshly collected as well as cultured mammalian cells exposed to non-ionizing radiofrequency fields (RF). These studies were reviewed and gaps in knowledge were identified [3,4]. The results from our more recent studies indicated that whole body of mice and cultured mouse bone marrow stromal cells (BMSCs) which were pre-exposed to 900 MHz RF at 120 μW/cm^2 power flux density for 4 h/day for few days showed significantly reduced levels of single and double strand breaks in the DNA as well as faster kinetics of their repair when subsequently challenged with bleomycin (BLM, a radio-mimetic chemotherapeutic drug) or gamma-radiation (GR) when compared with those that were not pre-exposed to RF [5,6]. These data suggested that DNA repair enzymes might have played a role and thus represent a potential mechanism for RF-induced AR.

There is well-documented evidence that poly (ADP-ribose) polymerase-1 (PARP-1), a family of nuclear enzymes in eukaryotic cells, is involved in several cellular functions including DNA repair, gene transcription, genomic instability, cell cycle progression and cell death. Among these nuclear enzymes, PARP-1 is more abundant and acts as a 'molecular nick sensor' to signal the cells about DNA strand breaks and to assist in their repair [7–13]. The results from our more recent preliminary experiments showed increased PARP-1 mRNA expression and its protein levels in BMSCs exposed to 900 MHz RF (continuous wave) at 120 μW/cm^2 power flux density for 3 h/day for 5 days indicating that RF exposure was capable of inducing PARP-1 [14]. Such increased PARP-1 might have played a role in reducing the DNA damage as well as its repair in BMSCs exposed to RF and subsequently

* Corresponding author.
E-mail address: yicao@suda.edu.cn (Y. Cao).
[1] These authors contributed equally to this work..

http://dx.doi.org/10.1016/j.mrgentox.2017.05.007
Received 26 October 2016; Received in revised form 18 April 2017; Accepted 11 May 2017
Available online 17 May 2017

challenged with GR [6] and thus, may have a role in RF-induced AR. Supporting evidence for this hypothesis comes from ionizing radiation-induced AR investigations. Increased PARP-1 mRNA expression and its protein level were observed in germ cells of mice and cultured mouse lymphoma EL-4 cells exposed to low dose ionizing radiation (IR) while treatment with 3-aminobenzamide (3-AB, a potent inhibitor of PARP-1) had negated such effects suggesting that PARP-1 had a role in cytogenetic and immune adaptive responses, respectively [15,16].

The current investigation was conducted with two specific aims. First, to confirm our preliminary observations of increased PARP-1 mRNA expression and its protein in BMSCs exposed to RF [14]. Second, to examine whether the induction RF-induced AR is negated if such cells are treated with 3-AB. Sham (SH)-exposed control cells and those exposed to 1.5 Gy GR (positive controls) were included in all experiments. RT-PCR technique for PARP-1 mRNA expression, Western blot analyses for PARP-1 protein and alkaline comet assay to examine single strand breaks and kinetics of their repair were used in the investigation.

2. Materials and methods

2.1. Bone marrow stromal cells (BMSCs)

The collection and culture of BMSCs were described in detail in our earlier paper [6]. Briefly, bone marrow cells from each of 4 adult male Kunming mice were obtained (protocol was approved by the Institutional animal care and ethics committee of Soochow university; approval number A68–2015). Single cell suspensions were prepared in complete IMDM medium (Iscove's modified Dulbecco's medium, Hyclone, Suzhou, China) containing 10% fetal bovine serum (FBS, Gibco, Shanghai, China), 100 units/ml penicillin and 100 μg/ml streptomycin (Bio Basic, Hangzhou, China). From each mouse, aliquots of $\sim 2 \times 10^5$ cells in 3 ml medium were placed in 60 mm petri dishes (Nunc, Shanghai, China) and cultured for 48 h in an incubator (Heal Force Bio-Meditech, Hong Kong, China) maintaining 37 ± 0.5oC with humidified atmosphere of 95% air and 5% carbon dioxide (CO_2). Then, the non-adherent cells were discarded in each petri dish and adherent cells were cultured further in fresh complete medium. Cells in 3–6 passages from a single mouse were used for different exposures described below. The entire experiment described below was repeated three times.

2.2. Radiofrequency fields (RF) and sham (SH) exposures

The exposure system was described in detail earlier [17]. Briefly, it consists of a GTEM chamber (Giga-hertz Transverse Electro-Magnetic chamber, 5.67 m length, 2.83 m width and 2.07 m height), a signal generator (SN2130J6030, PMM, Cisano sul Neva, Italy) and a power amplifier (SN1020, HD Communication, Ronkonkoma, NY). The 900 MHz RF continuous wave signal was generated, amplified and fed into the GTEM chamber through an antenna (Southeast University, Nanjing, Jiangsu, China). The RF field inside the GTEM was probed using a field strength meter (PMM, Cisano sul Neva, Italy) to determine the precise position which provided the required 120 μW/cm^2 power flux density used in the study. The rationale for using this power flux density was based on our earlier observation of a significant survival advantage of lethally irradiated mice which were pre-exposed to 900 MHz RF at 120 μW/cm^2 compared to those which were pre-exposed to RF at 12 μW/cm^2 or 1200 μW/cm^2 power flux density [18]. The power was monitored continuously and recorded every 5 min in a computer controlled data logging system during the 3 h RF exposure. The GTEM was installed in a room which maintained 37 ± 0.5 °C temperature (87% relative humidity, without CO_2) and the temperature inside the GTEM was similar during exposure of the cells.

For RF exposure, BMSCs in eight separate petri dishes (from a single mouse, arranged in two rows of 4 each and kept touching each other) were placed on a nonconductive table/platform at a height of 100 cm at the precise location where the required 120 μW/cm^2 power flux density was measured. The distance between petri dishes and the exposure unit (probe) was 18 cm. At the input 120 μW/cm^2 power flux density and the direction of propagation of the incident field parallel to the plane of the medium, the peak and average specific absorption rates (SARs) estimated were extremely low and were 4.1×10^{-4} and 2.5×10^{-4} W/kg, respectively [19]. BMSCs in eight other separate petri dishes were exposed in the GTEM chamber, without RF transmission and, these cells were used as SH-exposed control cells. The RF/SH exposure was 3 h/day for 5 days.

2.3. Gamma radiation (GR)

Cells in eight other petri dishes (which were left in the incubator) were exposed to an acute dose of 1.5 Gy GR (Nordion, Ottawa, ON, Canada, dose rate 0.5 Gy/min) from ^{60}Co source which was located in another building. There was an interval of ~10 min between GR exposure of the cells and their transport to the laboratory for use in different assays.

2.4. Experimental protocol

The experimental protocol is presented in Fig. 1. The entire investigation was repeated 3 times. The day before starting the investigation, aliquots of BMSCs from a single mouse ($\sim 5 \times 10^5$ cells/ml in 8 ml total) were seeded into several separate 100 mm petri dishes and were left in the incubator maintaining 37 ± 0.5 °C with humidified atmosphere of 95% air and 5% CO_2. Next day, before exposure, the medium was replaced with fresh medium in all petri dishes. Cells in eight petri dishes were used for each of the following 9 exposure conditions: unexposed control cells (for comet assay), SH, 900 MHz RF at 120 μW/cm^2 power flux density for 3 h/day for 5 days, acute 1.5 Gy GR alone, RF + GR, SH + GR, 3-AB alone, RF + 3-AB, RF + 3-AB + GR. There was an interval of 3 h between the last RF/SH exposure and GR. This interval was given to the cells to accumulate PARP-1, if any, induced by RF exposure and to see if the induction of PARP-1 was negated in cells treated with 3-AB. The cells in all petri

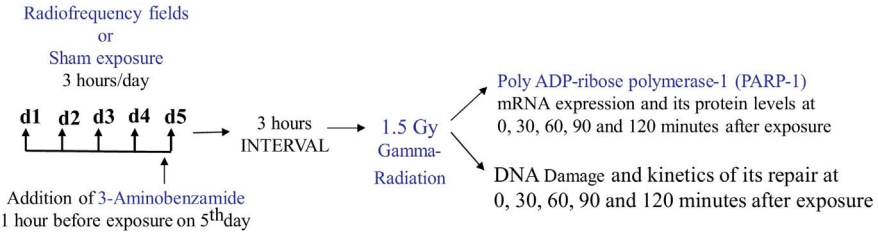

Fig. 1. Experimental protocol used in the study. Bone marrow stromal cells were exposed to continuous wave 900 MHz radiofrequency fields (RF) (or sham exposure, SH) for 3 h/day for 5 days. 3-aminobenzamide (3AB) was added to some cells at 1 h before exposure on 5th day. Three hours after the last exposure, cells were subjected to 1.5 Gy gamma-radiation (GR). Then, aliquots were used to detect poly (ADP-ribose) polymerase-1 mRNA expression and its protein level at 0, 30, 60, 90 and 120 min after exposure. An alkaline comet assay was used to assess DNA damage and its repair: one aliquot was used immediately and the kinetics of damage repair was evaluated at 0, 30, 60, 90 and 120 min after exposure.