Far Infrared Ray Irradiation Induces Intracellular Generation of Nitric Oxide in Breast Cancer Cells

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Received 15 Sep 2008; Accepted 25 Nov 2008

Abstract

Far infrared (FIR) radiation has been used in many health-promoting applications, but the cellular mechanisms have not been elucidated. We investigated the influence of non-thermal-enhanced FIR for generating nitric oxide (NO) in breast cancer cells. We used MCF-7 breast cancer cells treated with FIR irradiation or left untreated, and measured the inducible NO concentrations using the DAF-FM diacetate (4-amino-5-methylamino-2',7'-difluorofluorescein) technique. Mean fluorescence intensities of DAF-FM assays from different breast cancer cells showed progressive and cumulative increases in NO with FIR irradiation. Significant inductions of NO synthesis in breast cancer cells were observed both during and after FIR irradiation. Including data from a literature review, we discuss possible therapeutic roles of FIR for breast cancers through the induction of NO generation.

Keywords: Far infrared ray (FIR), Nitric oxide (NO), Breast cancer cells

1. Introduction

Far infrared (FIR) rays are short electromagnetic waves with wavelength ranges within the infrared spectrum. Most believe the optimal wavelengths of FIR for human biological effects range 8–14 μm. Although the exact mechanism is still not fully understood, they have been applied for many therapeutic purposes, including vascular-related disease and other health-promoting practices [1].

Physicists reported that FIR-treated water samples contain increased number of free tetrahedrals in clusters of smaller sizes. They observed that the collapse of the water clusters is initiated by absorption of the FIR spectrum, which may be converted into molecular vibrations [2]. It is believed that FIR induces both thermal and non-thermal effects, including an increase of microvascular dilation, a higher flow volume, and a slight elevation in the regional tissue temperature [3,4]. At the microscopic scale of heat transfer by subcutaneous tissues and other physical-biological processes, it is also believed that FIR can promote intracellular effects. Prior studies on FIR demonstrated that the increase in fibroblast proliferation could favorably impact wound healing [5], enhance immunity by strengthening leukocytes [6], and promote sleep [7,8].

With only limited published FIR research, there is an obvious lack of study of basic medical sciences and molecular biology. Two prior studies were conducted on FIR. The first was an animal experiment conducted by Hiroshi et al. [9], who used SHN female mice with mammary tumors. That study concluded the rate of tumor growth in these mice was inhibited after having received continuous exposure to FIR at a warm temperature. Another experiment was conducted by Yu et al. [10], which concluded that the biological effect of increasing skin microcirculation in rats was promoted by the arginine/NO pathway. NO is generated in the body through the transformation of arginine in citrulline into three isoforms of NO synthase (NOS). That study observed that the increase in skin microcirculation exerted by FIR could be suppressed by L-NAME (a type of NOS inhibitor).

In contrast to those two prior studies, we used human breast cancer cells instead of animal models. MCF-7 breast cancer cells were chosen because they are one of the most studied cell lines for NO induction, due to the high intracellular content of NOS, which in turn can more easily be detected. On the other hand, most prior studies used emission sources of FIR powered by electricity; so it might not be easy to exclude the thermal factor on the biological effects. In our current study, we used a simple FIR source at room temperature to eliminate the external-heat effect, and

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concluded that non-thermal-enhanced FIR irradiation can induce the generation of intracellular NO.

2. Materials and methods

2.1 FIR ceramic powder

The ceramic powder consisted of micro-sized particles produced from several ingredients, mainly mineral oxides. The average emissivity of the ceramic powder was 0.98 at wavelengths of 8–14 μm, which was proven by tests run by the Industrial Technology Research Institute, and this represented an extremely high ratio of FIR ray intensity. Equal amounts of 100 g of FIR powder (FIR groups) and nonfunctional milk powder (control groups) were enclosed in 10 different plastic bags.

2.2 Cell culture for FIR irradiation

The MCF-7 human breast cancer cell line, (estrogen receptor-positive and progesterone receptor-positive cells) of a low-grade malignant cell type, was grown in a suspension and incubated at 37°C in a 5% CO_2 atmosphere in the dark until cells covered about 80% of the bottom of ten incubators. We inserted five plastic bags filled with FIR powder (as the FIR group) and five plastic bags filled with non-functional powder (as the control group) beneath the dishes containing breast cancer cells, which were then irradiated by FIR without direct contact (Fig. 1).

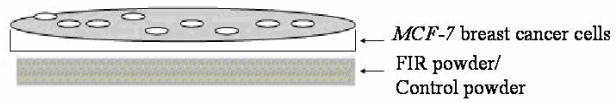

Figure 1. Cell culture. FIR powder (as the FIR group) and non-functional powder (as the control group) were inserted beneath dishes containing breast cancer cells.

2.3 Flow cytometric measurements

Ten dishes with FIR powder (as the FIR group) and nonfunctional powder (as the control group) were divided into five categories: group 1–3, placed for 0-, 10-, and 60-minute intervals of treatment with the powder-containing bags; and group 4 and 5, placed for 10 and 60 minutes respectively and then the powder-containing bags were taken away for 90 minutes (Table 1). All dishes were then stained with DAF-FM diacetate for fluorescence measurements. All cells were analyzed by a fluorescence-activated cell sorter (FACS) and flow cytometry at the single-cell level. As the data were acquired and analyzed, the mean fluorescence intensities of the breast cells were determined. Different groups and categories of fluorescence in these cells were recorded on intensity profiles and mean histograms.

2.4 Statistical analysis

Statistical significance between the control and FIR groups was determined by unpaired *t*-test. A value of $p < 0.05$ was considered statistically significant.

Table 1. Treatment with FIR irradiation.

Group	Treatment
1	FIR irradiation at the beginning
2	FIR irradiation for 10 minutes
3	FIR irradiation for 60 minutes
4	FIR irradiation for 10 minutes, and separation for 90 minutes
5	FIR irradiation for 60 minutes, and separation for 90 minutes

3. Results

Levels of NO synthesis in the control and FIR groups are listed in Table 2. Figure 2 compares the NO production of the control and FIR groups. The effects of different treatment times are shown in Fig. 3. Figure 4 demonstrates an obvious increase in NO production due to FIR treatment for 10 minutes, and then there is a cumulative and further increase up to 90 minutes. Readings of the mean fluorescence intensity in different categories of breast cancer cells (Figs. 2–4) showed progressive and cumulative increases in NO in the FIR groups compared to the control groups. The *p* value between these two groups was 6.63×10^{-4} ($p < 0.05$), which shows a significant difference (Table 2). This result demonstrates that FIR can induce NO synthesis in breast cancer cells. In addition, there were increases of 37.5% in NO generation after 10 minutes and 50.0% after 60 minutes of FIR irradiation. There were further increases to 50.0% and 62.5% at 90 minutes after respectively completing 10 and 60 minutes of FIR irradiation (the post-FIR effect) (Table 2).

Table 2. NO synthesis in the control and FIR groups.

Group / Irradiation time (minutes)	Control	FIR (cumulative increase % of NO)
0	70	80 (0%)
10	70	110 (37.5%)
60	60	120 (50.0%)
10 then 90 minutes of separation	55	120 (50.0%)
60 then 90 minutes of separation	60	130 (62.5%)
p value	6.63×10^{-4}	

4. Discussion

An animal experiment was conducted by Hiroshi et al. [9], who used SHN female mice with mammary tumors [11]. That study concluded that the size of mammary tumor growth was inhibited significantly in the FIR group compared with the control group (continuous FIR exposure for 16 days with the FIR source surface maintained at 40°C). In addition, the study demonstrated that mice in the FIR group exhibited a lower level of epidermal growth factor receptor (EGFR) than did the control group [9,12]. EGFR is believed to be essential in the production of cell surface receptors that bind to transforming growth factor-alpha (TGFα), which participates in breast carcinogenesis [13].

NO has a short half-life of a few seconds and is rapidly oxidized to stable and inactive end products, including nitrite

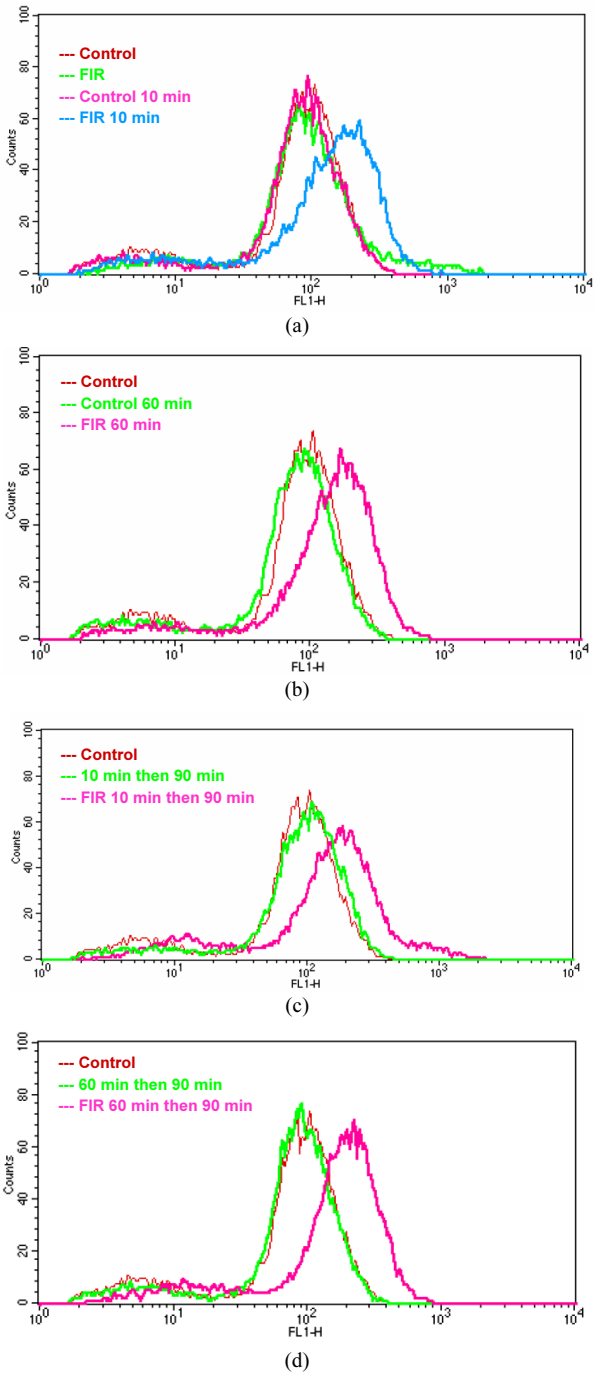

Figure 2. Comparison of the control and FIR groups in NO production by flow cytometric analysis; (a) remarkable increase in the concentration of NO after 10 minutes of FIR irradiation; (b) remarkable increase in the concentration of NO after 60 minutes of FIR irradiation; (c) remarkable increase in the concentration of NO after 10 minutes of FIR irradiation and separation for 90 minutes; (d) remarkable increase in the concentration of NO after 60 minutes of FIR irradiation and separation for 90 minutes.

and nitrate. It causes different physiological and pathophysiological impacts. Although NO is produced by a number of cancer cell lines and other solid tumors, the exact role of NO in cancer biology is still not fully understood [14,15].

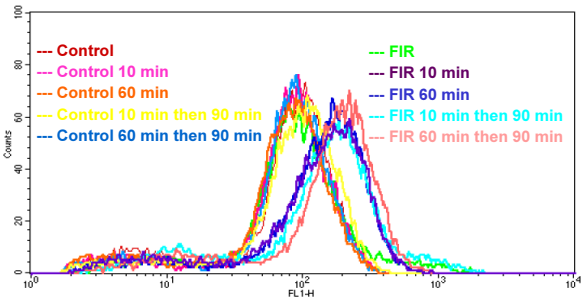

Figure 3. Comparison of the control and FIR groups with different treatment periods.

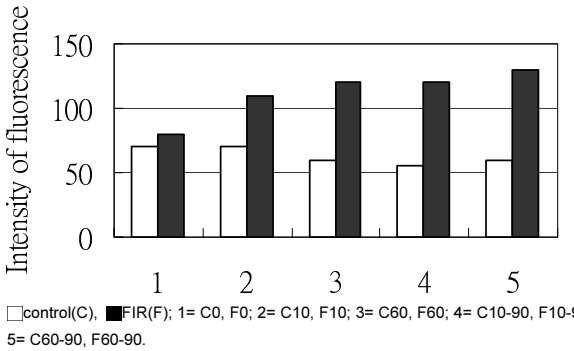

Figure 4. The mean intensity of the fluorescence (NO count) in different treatments.

Lahiri et al. [14] demonstrated a significant decrease in NO production in breast cancer with multidrug resistance and estrogen receptor-negative cell type (MCF-7 ADR) compared to the drug-susceptible cell type (MCF-7).

NO is a physiological secretory product of normal breast cells, which is involved in some critical functions of homeostasis and host defense [16]. Another previous study demonstrated that normal breast cells showed 100% staining for NOS [14,17], which is responsible for producing NO. Also, there was a reduced percentage of positive staining of NOS in those breast cancer cell types with increasing characteristics of malignancy (percentage positively stained for NOS: normal mammary tissue > ZR-75-1 > ZR-75-9a1 > ZR-PR-LT) [17]. Although many kinds of breast cancer cells have been shown to produce NO *in vivo* and *in vitro*, it was reported that a higher expression of NOS was produced in estrogen receptor-positive ZR-75-1 cells (a breast cancer type with low-grade malignancy and high drug susceptibility) compared to the lower expression of NOS in a multidrug-resistant cell line [14,18].

Similar results were observed by Reveneau et al. [19], who reported that an immunohistochemical study of cellular proliferation revealed that breast tumors with low proliferation rates had high NOS activities. Those results were reinforced by their *in vitro* observation that NO inhibited the proliferation of human breast cancer cells, which explains the relationship between the higher NO production and the weaker tumor aggressiveness. Based on a review of these prior studies, we believe that the reduction in NOS activity and decrease in NO production correlate with the characteristics of tumor aggressiveness. In addition, breast cancer cell lines with a

higher NOS activity have a lower proliferative rate and a lower malignant grade; thus, NO seems to act *in vitro* as an inhibitory factor of carcinogenesis in human breast cancer cells [19]. Apart from the breast cancer cell line, it was also reported that non-metastatic K-1735 melanoma cells exhibited a higher level of NOS, in contrast to metastatic cells [20-22].

The purpose of this study was not to predict whether the carcinogenesis of breast cancer cells could be inhibited by FIR irradiation itself, but through the generation of intracellular NO, FIR probably restored some normal breast cell characteristics of a high-grade breast cancer. This may provide new hope of combining hormones and chemical therapy with FIR, as FIR may increase drug susceptibility. Our study attempted to ascertain the effects of FIR irradiation at room temperature without an electricity source, with the help of advanced materials technology. This method could induce the intracellular generation of NO. These experimental data showed a rapid response of NO production of as short as 10 minutes, which supports the previous suggestion [10] that maintaining a biological effect with post-FIR irradiation and a heat supply can last as long as 90 minutes. Based on this result, we suggest that more basic medical studies be conducted on non-thermal-enhanced FIR emission sources and the related therapeutic values in oncology and other medical fields as well. Finally, we found that DAF-FM diacetate assay measured with the flow cytometry is an accurate, fast, and relatively easy technique to detect the cellular generation of NO induced by FIR.

Acknowledgments

The authors gratefully acknowledge the support provided to this study by Mr. Francis Chen (Franz Collection Inc.) and Dr. Shawn Huang (Purigo Biotech Inc.).

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