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Short Communication

Exploring the promising therapeutic benefits of iodine and radioiodine in breast cancer cell lines

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Abstract

Iodine has an anti-proliferative effect on cancer cells; however, its effects have not been explored adequately. The aim of this study was to evaluate the therapeutic potential of iodine and radioiodine by assessing their effects on the viability of various breast cancer cell lines: MCF7, SKBR3, and MDA-MB231. The viability of cells was measured in treated cells exposed to six doses of iodine (5, 10, 20, 40, 60, 80 µM) and two doses of radioiodine (3.7×10^4 and 3.7×10^5 Bq). A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and modified clonogenic assays were used to assess cell viability. Exposure to 80 µM of iodine significantly reduced the viability of all cell types. The cells were then exposed to a 50% inhibitory concentration (IC_{50}) dose. When the cells were exposed to the IC_{50} dose of iodine, the MCF7 cell viability was reduced by 42.6±0.14% (IC_{50} dose 12.88 µM), 40.2±0.08% for SKBR3 (IC_{50} dose 11.03 µM) and 47.0±0.02% for MDA-MB231 (IC_{50} dose 14.09 µM). All cells were also exposed to 3.7×10^4 Bq and 3.7×10^5 Bq radioiodine. Both doses significantly reduced the cell viability of MCF7 and SKBR3 cells compared to the unexposed control cells (all had $p<0.05$), while MDA-MB231 cell viability only reduced significantly after 3.7×10^5 Bq of radioiodine exposure compared to the unexposed control cells ($p<0.05$). This study highlighted that iodine had a toxic effect on breast cancer cells, and radioiodine enhanced the toxicity to breast cancer cells. The types of cancer cells and doses of iodine and radioiodine influenced the effect. These findings suggest that iodine and radioiodine hold promise as therapeutic agents for breast cancer, similar to their established use in thyroid disease treatment. However, further in vivo studies are important to provide more evidence.

Keywords: Adjuvant therapy, cell viability, HER2+, luminal A, triple-negative

Introduction

Breast cancer is the most common malignancy among women worldwide, according to the Global Cancer Observatory of Breast Cancer in 2020 [1]. New cases of breast cancer in 2020 were estimated to be 2,261,419 (11.7%), with an estimated 684,996 (6.9%) deaths [1]. In Indonesia, breast cancer ranks as the third cause of death after lung and liver cancers [2]. Breast cancer care, in particular, is associated with a high financial burden given the need for screening and diagnosis, multidisciplinary care, and longitudinal follow-up and costs approximately 171 billion dollars in the USA annually [3].

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Breast cancer subgroups depend on the receptors possessed by cancer cells, and these include estrogen receptor (ER), progesterone receptor (PR), and human epithelial receptor 2 (HER2) [4,5]. These subgroups include luminal A (characterized by estrogen and progesterone receptors (ER+/PR+) and HER2 negative (HER2-), luminal B (ER+/PR+ and HER2+), HER2 positive (absence of ER and PR receptors (ER-/PR-) and HER2+), and basal-like or triple negative (ER-, PR-, and HER2-) [6]. Approximately 70% of breast cancers express estrogen receptor (ER+), and patients with ER+ are treated with anti-hormone therapies such as tamoxifen [6-7]. Of the patients who received tamoxifen, 30% experienced a recurrence within the next decade and developed resistance after prolonged exposure to the drug, especially in the metastatic setting [8]. On the other hand, HER2+ and triple negative B (TNB) subtypes have a poorer therapeutic response and prognosis than breast cancer, which expresses ER+ or PR+ [6-9]. Overexpression of the oncogene HER2+ activates growth factor and induces uncontrolled proliferation, and the HER2+ subtype does not respond to hormonal treatment and has a high probability of relapse and metastasis [7,10]. Moreover, the TNB subtype does not respond well to hormonal therapy, has the worst prognosis and exhibits the fastest cell proliferation [10].

Iodine has anti-proliferative effects that can suppress the growth of neoplastic cells in some organs, including the breast [11,12]. Iodine targets mitochondria in cancer cells by depleting thiol reserves and disrupting the mitochondrial membrane potential, leading to the activation of apoptotic pathways [13]. Additionally, it inhibits tumor growth by producing 6-iodolactone (6-IL) through the iodination of arachidonic acid [13]. This iodolipid functions as a strong ligand of peroxisomal-activated receptor type gamma (PPARγ), facilitating re-differentiation by inhibiting stem signaling and initiating apoptosis [13].

Iodine uptake by breast cancer cells is independent of the natrium iodide symporter (NIS) [14,15]. It is an essential element needed by the body, especially the thyroid and breast, and plays a role in synthesizing thyroid hormones that function in the growth and metabolism of cells in some organs [11,16]. Iodine deficiency stimulates abnormalities in the breast or thyroid organs, resulting in atypic, dysplasia, or hyperplasia [16,17]. Iodine has been shown to reduce hyperplasia in rat breast cells and per lobular or ductal fibrosis [15]. Iodine reduces cell proliferation through direct antioxidation and indirectly by activating PPARγ receptors, triggering apoptosis and cell redifferentiation [18,19]. The cell proliferation inhibitory effect of iodine has been shown to be dose-dependent in breast cancer [20-22].

Radioiodine, a radioactive isotope of elemental iodine, is commonly used to treat hyperthyroidism and thyroid cancer. It emits negatively charged beta and gamma (β and γ) rays. The β particle has a range of ~3 mm in the tissue and is used for therapy, while the γ ray is used for imaging [23-25]. The β particle inhibits cell growth by inducing cell death directly or indirectly by hitting and degrading DNA [23-25]. These facts open an opportunity to use radioiodine for breast cancer therapy, especially for those who are not responsive to conventional therapy. The aim of this study was to assess the therapeutic potential of iodine and radioiodine in some breast cancer cell lines by measuring cell viability.

Methods

Cell culture

The study used three breast cancer cell lines: MCF7, SKBR3, and MDA-MB231. They represent luminal A, HER2+, and TNB subtypes, respectively. The SKBR3 cell line was obtained from the American Type Culture Collection (ATCC) while MCF7 and MDA-MB231 were received from the Faculty of Medicine, Universitas Padjajaran, Bandung, Indonesia. The SKBR3 cell line was cultured in McCoy's 5A medium (HiMedia, Kennett Square, USA, #AL057), and 1640 RPMI medium (Corning Inc., Corning, USA, #10-040cv) was used for MCF7 and MDA-MB231 cell cultures supplemented with 1% fetal bovine serum (Gibco, Grand Island, USA, #10270098), 1% penicillin, 1% streptomycin, and 1% amphotericin B (HiMedia, Kennett Square, USA, #A002) [5]. Cells were grown until 90% confluency and then trypsinized and washed with phosphate-buffered saline (PBS) twice before being replanted for treatment.

Iodine treatment

The cells were plated in a 96-well plate at a density of 2×10^4/well and incubated for 24 hours. The cells were treated with iodine (Sigma-Aldrich, Burlington, USA, #207772) dissolved in warm aquabidest. Six doses (5, 10, 20, 40, 60, 80 µM) were used to treat the cells for 72 hours [26,27]. A control cell was treated with medium only. The half-maximal inhibitory concentration (IC_{50}) values were calculated using linear regression. The absorbance was measured using a spectrophotometer, and the experiments were conducted in three replications.

Cell viability

Cell viability was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and modified clonogenic assay. During the MTT assay, the cell medium was replaced with a fresh medium, and the cells were incubated with 10 µL of 12 mM MTT solution for 4 hours at 37°C. A total of 50 µL of dimethyl sulfoxide was added to each well, and the cells were then incubated for 10 mins at 37°C. The absorbances were measured using a spectrophotometer, and the cell viability in percentage (%) was calculated as the optical density (OD) of the tested divided by the OD of the control, multiplied by 100% [28].

A modified clonogenic assay was also used to assess cell viability. The breast cancer cell lines were plated in 25 cm^2 tissue culture flasks. The cells were grown in 5% CO_2 at 37°C until 80% confluence. They were treated with an IC_{50} of iodine for 72 hours. The cells were washed twice with cold Hank's balanced salt solution (HBSS). The cells were then cultured for 12 days in a medium supplemented with 10% fetal bovine serum, 1% penicillin, 1% streptomycin, and 1% amphotericin B. The breast cancer cells were washed twice with PBS, fixed with methanol/water (1:1), and stained with crystal violet, which stained the cells purple. Cell viability was analyzed by measuring cell density and the proportion of purple stained area on the microscopic image, which was reported in percentage area. The purple-stained areas were isolated using the ImageJ 1.49v program (National Institute of Health and Laboratory for Optical and Computational Instrumentation, Bethesda, USA).

Radioiodine treatment

The cells were plated on a 96-well plate at a density of 2×10^4/well and incubated for 24 hours [5]. They were then treated with two doses of sodium iodide-131 (NaI-131): (A) 3.7×10^4 Becquerel/mL/well (1 µCi/mL), and (B) 3.7×10^5 Becquerel/mL/well (10 µCi/mL), for 7 hours in 5% CO_2 at 37°C with 10mM HEPES (pH 7.3). The control group was incubated with HBSS only (Gibco, Grand Island, USA, #14025092). The absorbance was measured using a spectrophotometer at a wavelength of 570nm. The cell viability in percentage (%) was calculated as the OD of the tested divided by the OD of control, multiplied by 100% [28].

Data analysis

To compare the cell viability among groups, ANOVA, Kruskal-Wallis and Mann-Whitney tests were used as appropriate with normal distribution. The IC_{50} values were calculated using linear regression. The IC_{50} value was determined by the linear regression equation derived from the concentration versus proliferation percentage graph. The data were analyzed using SPSS23 (IBM, Armonk, USA).

Results

Cell viability post-exposure to iodine

The impacts of iodine exposure on the viability of breast cancer cells are illustrated in **Figure 1**. The viability of MCF7 cells, indicative of luminal breast cancer, remained relatively stable at doses ranging from 5–40 µM (91–99%). However, at 60 µM, viability decreased to 87%, and at 80 µM, it reduced significantly to 42.6% (**Figure 1A**). The HER2+ type of breast cancer cell line, SKBR3, showed significantly lower cell viability (40.2%) after treatment with 80 µM iodine compared to the control group. Cell viability remained stable (90.03–100%) at doses of 5–60 µM and was not different compared to the untreated group (**Figure 1B**). The TNBC cell line, MD-MB231, showed a similar response with MCF7 and SKBR3 cells. The viability was not significantly decreased at

doses of 5–60 µM (91.79–83.37%); however, at 80 µM, the viability reduced to 47.0%, significantly lower compared to the untreated control group (p=0.037) (**Figure 1C**).

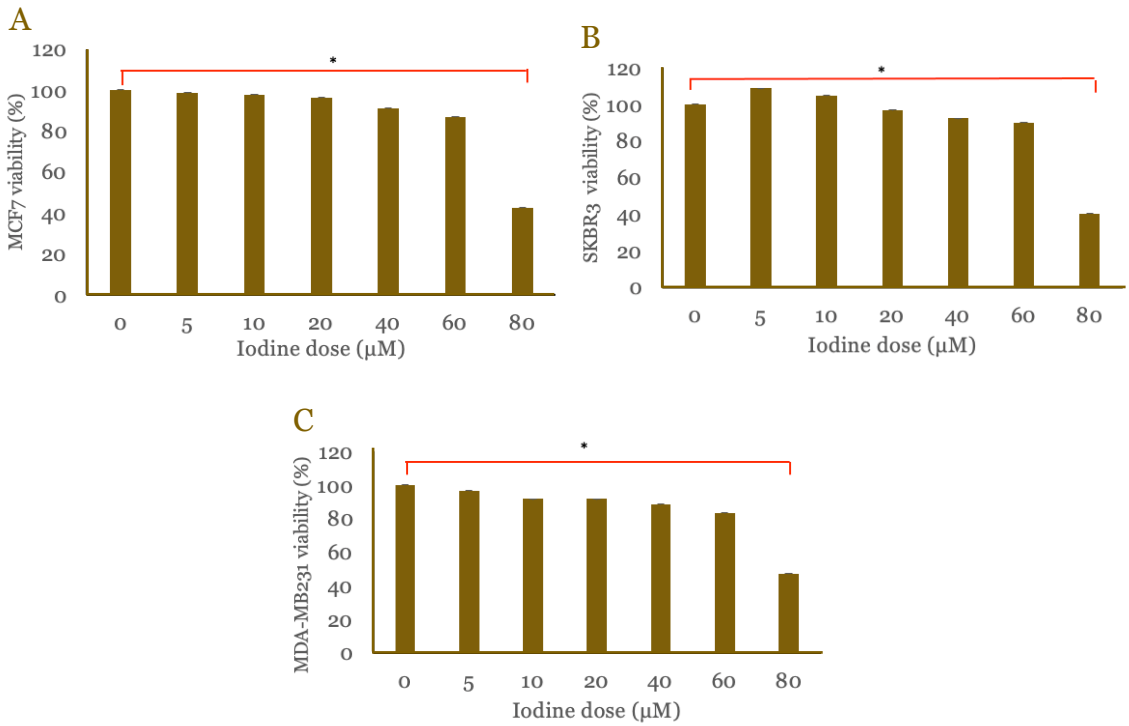

Figure 1. The cell viability was reduced in all cell lines post-treatment of 80 µM iodine. (A) The viability of MCF7 cells after treatment was 42.6±0.14%, compared to control cells; (B) The viability of SKBR3 cells after treatment was 40.2±0.08%, compared to control cells; (C) The viability of the MDA-MB231 cell after treatment was 47.0±0.02%, compared to the control cell. The asterisk * indicates the comparison is statistically significant at p<0.05.

Cell viability post-exposure with IC_{50} iodine

The cells were exposed to doses corresponding to IC_{50} and allowed to grow for 12 days. The cell viabilities were reported based on the proportion of cell density area, summarized in **Figure 2**. The MCF7 cells were treated with 12.88 µM of iodine. The means of cell viability were 62.60±14.14% and 49.25±5.30% of control and treated cells, respectively, p>0.509 (**Figure 2A**).

The SKBR3 cells exhibited a reduction in cell viability with its IC_{50} iodine (11.03 µM), although not significant compared to unexposed cells. The means of cell viability were 14.95±6.29% and 2.75±0.64% of control and treated cells, respectively, p=0.121 (**Figure 2B**). Following exposure to the IC_{50} iodine (14.09 µM), the MDA-MB231 cells showed viability of 69.05±3.75% and 27.80±5.66% of control and treated cells, respectively, p=0.121 (**Figure 2C**).

Cell viability post-exposure with radioiodine

The MCF7 cell viability decreased to 78.74±8.07% and 6.45±2.72% by exposing to 3.7×10^4 Bq, and 3.7×10^5 Bq of radioiodine, respectively, p<0.001. Both doses significantly reduced the cell viability compared to the control cell with p=0.006 and p<0.001, respectively (**Figure 3**).

The viability of SKBR3 cells reduced to 10.10±1.7% and 1.33±0.57% after exposure to radioiodine at 3.7×10^4 Bq and 3.7×10^5 Bq, respectively, p<0.001. Both doses reduced the viability of SKBR3 cells compared to control cells, with p<0.001 for both. The viability of MDA-MB231 cells after 3.7×10^4 Bq reduced the cell viability to 79.33±14.86%, and exposure with 3.7×10^5 Bq dose of radioiodine reduced the cell viability to 15.08±6.87%. The cell viability was significantly reduced after the cells were treated with 3.7×10^5 Bq of radioiodine compared to 3.7×10^4 Bq dose and control, with p<0.001 for both. However, the viability of the treated cells with 3.7×10^4 Bq of radioiodine was not significantly different from the control group, p=0.110 (**Figure 3**).

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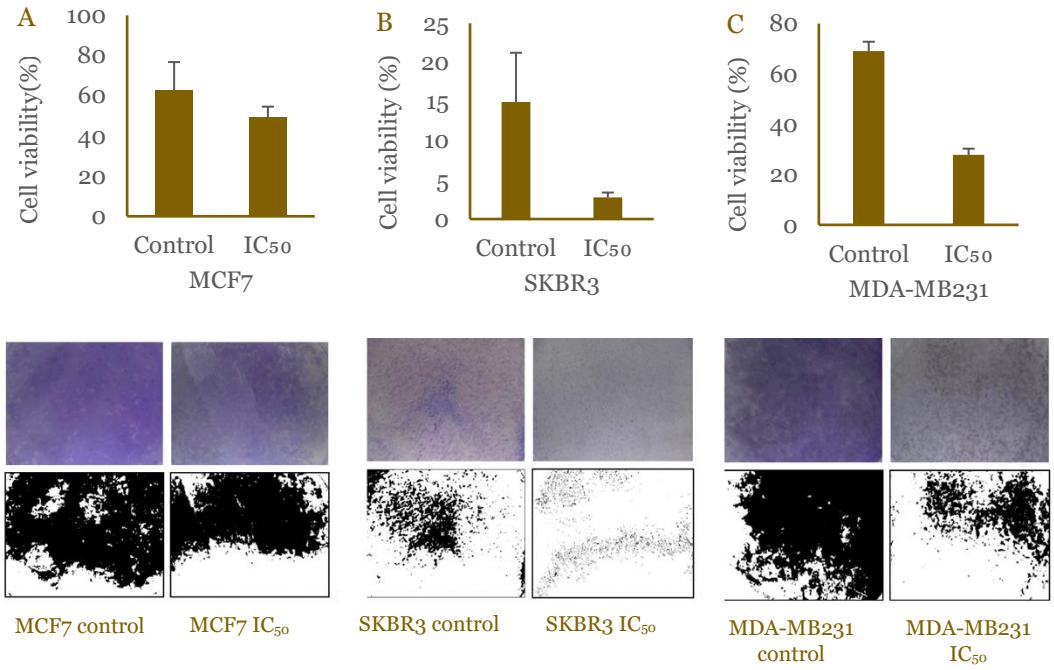

Figure 2. The cell lines (MCF7, SKBR3, and MDA-MB231) were exposed to iodine with a concentration of IC_{50} dose for twelve days. The effects of the treatments on the viability of MCF7 cells (A), SKBR3 (B), and MDA-MB231 (C) were assessed and compared to control. The results showed that the cell viability of MCF7, SKBR3, and MDA-MB231 reduced even though statistically insignificant for all cells. Bottom panel shows the cell density from stained areas with the ImageJ program.

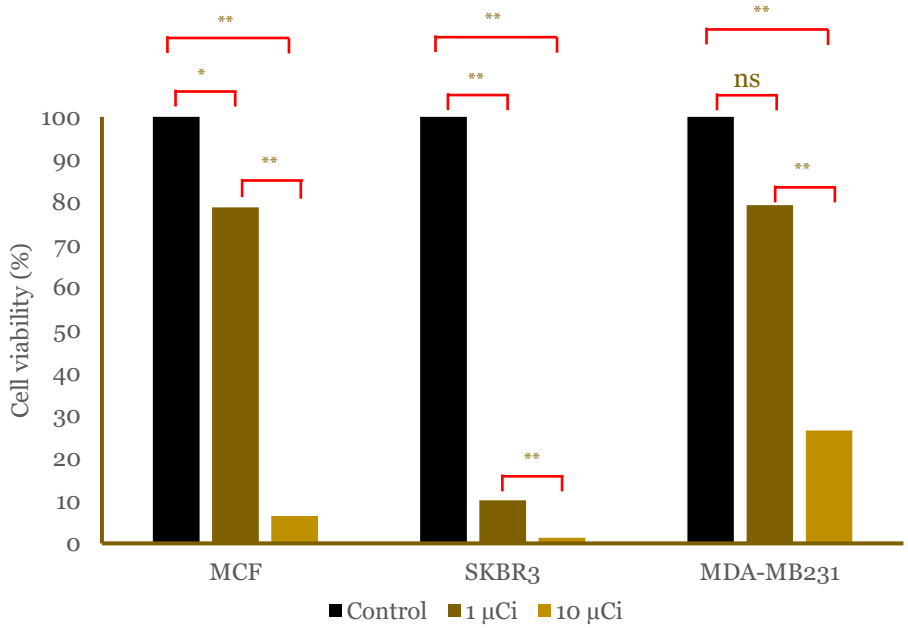

Figure 3. Comparison of cell viability post-exposure with different doses of radioiodine. The cells were exposed to two doses of radioiodine (3.7×10^4 Bq/well (1 µCi) and 3.7×10^5 Bq/well (10 µCi) for seven hours. The radioiodine dose of 3.7×10^5 Bq significantly reduced the viability of all three cells compared to 3.7×10^4 Bq/well dose. ns: not significant. The asterisks * and ** indicate the comparison is statistically significant at $p<0.05$ and $p<0.001$, respectively.

Discussion

Iodine is an essential element required for the biosynthesis of thyroid hormones and regulation of metabolic pathways in body organs [29,30]. Iodine is associated with thyroid and breast cancer, where the deficiency or excess supplementation may promote the cell proliferation rate

of thyroid cancer cells, and the condition, combined with tumor-inducing factors, involves a direct carcinogenesis pathway at the DNA level [16,31]. Iodine deficiency may stimulate a hyperestrogenic state, increasing breast cancer risk [11,12,32]. A study reported that breast cancer risk was 25% lower in women with high iodine levels than those with low levels, particularly among women with high selenium levels [33]. Molecular iodine is a micronutrient that exhibits antineoplastic properties related to breast cancer. Its mechanisms of action involve direct antioxidant effects, such as scavenging reactive oxygen species (ROS) and regulating mitochondrial function. Additionally, it exerts indirect effects by activating PPARγ receptors, initiating apoptosis, and promoting cell redifferentiation [13]. In this study, the cells were exposed to iodine at a concentration of 80 μM for 72 hours resulted in toxicity to all examined breast cancer cell lines. In contrast, lower concentrations did not exhibit any toxic effects on the cells (**Figure 1**). Moreover, after the treated cell with IC_{50} dose was planted for a period of 12 days, the cell viability was reduced, even though statistically insignificant (**Figure 2**). Further in vivo studies are needed to explore the effect of the iodine.

It has been established that the uptake of iodine in non-lactating breast cells occurs independently of NIS, Pendred Syndrome (PDS) gene, and Na+/K+ ATPase, strongly suggesting the involvement of a diffusion system in its transport [22,23]. The comprehensive in vitro studies have established that iodine is taken up by MCF7 cells regardless of NIS expression and significantly inhibits their proliferation [20,21]. In this study, the MCF7 cell viability was significantly reduced after exposure to both radioiodine doses, even though MCF7 cells did not express NIS. On the other hand, the expression of NIS in HER2+ and TN subtypes of breast cancer has been consistently reported, with a significant 19.5% of breast cancer cells exhibiting functional NIS expression [5]. Our study found that the viability of SKBR3 cells was reduced significantly with both radioiodine doses. However, the MDA-MB231 cells reduced the viability significantly for the cells treated by 3.7×10^5 Bq compared to 3.7×10^4 Bq and control cells only (**Figure 3**).

The heterogeneity of breast cancer makes it difficult to treat, particularly for patients with HER2+ and TN subtypes. The ability of breast cancer cells to take up iodine has led to the hypothesis that radioiodine could be a potential treatment for breast cancer, and radioiodine needs to reside in breast cancer cells for therapeutic purposes [5,34,35]. Moreover, it might be helpful for breast cancer luminal A type, which resists hormonal therapy. A full understanding of iodide transport and breast cancer response based on subtypes is needed for applying radioiodine as an alternative therapy for breast cancer. In our study, the iodine demonstrated cell growth inhibition and cytotoxic effects in a significantly dose-dependent manner and time exposure. Other studies reported similar findings, aligning with our results [11,20,22,27,36].

Furthermore, iodine has antioxidant, antineoplastic, and apoptotic effects in several cancer cells [30,33]. Antineoplastic and immunomodulatory effects of iodine suggest at least two mechanisms: a direct action of its antioxidant/oxidant properties and an indirect effect through iodolipid formation [30]. Although the mechanism of inhibitory cell proliferation was not examined in this study, the effect of iodine was suggested to be associated with arachidonic acid (AA), which can inhibit the growth of breast cancer cells, that can be attributed to the PPAR pathway, even though there are controversial views that AA stimulates breast cancer proliferation in a time and dose-dependent manner [37]. Iodine inhibitory activity might be generating 6-IL through the AA iodination [13,25]. The 6-IL concentration increased 15 times after iodine supplementation in normal mammary tissue and the MCF7 cell line [30]. The 6-IL is a specific ligand and a potent promoter of PPAR. These receptors are ligand-activated transcription factors associated with redifferentiation by inhibiting stem signaling and triggering apoptosis [13,30].

Iodine has antioxidant, antineoplastic, and apoptotic effects in several cancer cells. It also exhibits modulatory properties in the immune system [30]. Additionally, radioiodine can modify cancer cell phenotypes and enhance the effect against cancer cells [37]. It can be hypothesized that various immune cells can internalize radioiodine, and the cell response depends on the cellular context.

Radiodine is a radioactive isotope of elemental iodine that is used as adjuvant therapy for thyroid cancer. It emits negatively charged β particles, which inhibit cell growth by inducing cell death directly or indirectly by hitting and degrading DNA [23-25]. Antiproliferative effect of

iodine robust by the β particle of radioiodine emits. The response of the cells is different for each breast cancer subtype, and also depends on doses administered. Besides the types of breast cancer cells and the dose, it is important to note that the duration of exposure to radioiodine in this study was limited to 7 hours, while the half-life of radioiodine is approximately eight days, which may have contributed to the observed results. The prolonged exposure may yield different outcomes in cell viability. Our study provides evidence that iodine and radioiodine have the potential to reduce breast cancer in vitro. This highlights the need for further in vivo studies to advance their clinical application in breast cancer therapy. This is especially crucial for treating breast cancer cases that do not respond to existing treatments.

Conclusion

Iodine and radioiodine exhibit a dose-dependent capacity to reduce the viability of breast cancer cells in vitro. Both iodine and radioiodine appear to decrease the viability of all three breast cancer subtypes. These findings indicate that further investigation is warranted to explore the potential role of iodine as an enhancer and radioiodine as an adjuvant therapy in breast cancer treatment, especially for subtypes that do not respond to current therapies.

Ethics approval

Ethical approval is not required for this study.

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Competing interests

The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

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