Dose-Response:
An International Journal
October-December 2019:1-6

DOI: 10.1177/1559325819896183
journals.sagepub.com/home/dos
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Quantitative Evaluation of Hormesis in Breast Cancer Using Histoculture Drug Response Assay

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Abstract

Purpose: Hormesis is a phenomenon of growth stimulation at low doses and inhibition at higher doses. In cancer treatment, little is known about how hormesis affects cancer cell proliferation. We evaluated the hormetic dose–response relationship of paclitaxel using surgically resected breast cancer specimens on the basis of histoculture drug response assay (HDRA).

Methods: We used surgically resected fresh tumor specimens from 22 patients with breast cancer: 17 invasive ductal, 3 mucinous, and 2 other "special-type" cancers. All patients were female, ranging in age between 40 and 86 (median 60) years. Small pieces of viable cancer tissue were placed on collagen gel and cultured for 7 days with paclitaxel. Inhibition rates of paclitaxel at several concentrations were measured and fitted to a sigmoid dose–response curve.

Results: Hormesis was observed in 9 of the 22 cases; ED_{50} of cytotoxic effect was significantly higher ($P = .0036$) in hormesis (H) group (44.6 $\pm$ 4.2 μg/mL) than in nonhormesis (N) group (26.7 $\pm$ 3.5 μg/mL).

Conclusion: We evaluated hormesis in breast cancer tissue using HDRA for the first time although previously confirmed in cultured cells. Hormesis seems to occur in patients undergoing treatment with anticancer agents, especially in a metastatic setting. Meanwhile, tumor growth may be stimulated in patients who are resistant to paclitaxel.

Keywords
hormesis, dose–response curve, histoculture drug response assay, breast cancer

Introduction

In breast cancer treatment, chemotherapy plays an important role in both (neo)adjuvant and metastatic settings. In (neo)adjuvant setting, dose-intensity of chemotherapy, especially maximum tolerated dose, correlates well with disease-free survival. For patients with triple-negative breast cancer, this leads to frequent application of dose-dense chemotherapy. In the metastatic setting, the aim of chemotherapy is to alleviate or prevent unpleasant symptoms caused by the metastatic breast cancer, so dose delay or dose reduction of chemotherapy is often allowed in order to maintain patient quality of life.

Hormesis is a phenomenon in which a cell or organ exhibits a biphasic response to a chemical agent (eg, digoxin) or to an environmental factor (eg, alcohol and ionizing radiation).[1-3] Within the hormetic zone, there is a favorable biologic response (eg, antiaging effect) to low-dose toxins and other stresses.[4] Hormesis may also exhibit a potentially adverse impact on cancer treatment with chemotherapeutic agents, however, which can often induce oxidative stress in cancer cells. Low-dose cytotoxic agents may therefore induce hormesis to stimulate cancer cell proliferation. Cultured human cancer cells have shown hormesis at low doses of various anticancer agents, which is then followed by inhibition at higher doses of the same agents.[5,6]

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Received 27 August 2019; received revised 25 November 2019; accepted 26 November 2019

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Table 1. Patient Characteristics.

N	Breast cancer	22
Age (median), years	40-86 (60)	
Gender	Female	22
	Male	0
Histologic type	Invasive ductal carcinoma	17
	Special type	5
Nuclear grade	1, 2	15
	3	7
Hormone receptor status	Positive	20
	Negative	2
HER2 status	Positive	6
	Negative	16

Abbreviation: HER2, human epidermal growth factor receptor type 2.

The histoculture drug response assay (HDRA) using fresh tumor tissue has been established to evaluate chemosensitivity to a given chemotherapy agent. The tumor inhibition rates (IRs) of chemotherapy agents using HDRA have been well-correlated to clinical response in various types of cancer. The correlation rate between IRs of HDRA and clinical response was reported to be 77.8% to 92.1% in head and neck, breast, lung, ovarian, and colon cancer.[7-11] However, the effects of hormesis have not yet been investigated.

In this report, we examine whether hormesis exists in breast cancer treatment. We evaluate the hormetic dose–response relationships of anticancer agents for breast cancer using HDRA.

Patients and Methods

Patients

To analyze the hormetic dose–response relationship of paclitaxel, surgically resected fresh tumor specimens were obtained from 22 women with breast cancer. Patient characteristics are shown in Table 1. The study was approved by the Wakayama Medical University Hospital Institutional Review Board (2383). This is retrospective research that uses the information on HDRA obtained from a previous study.[12] It was therefore sufficient to post the study information at the site of the related facilities without necessity for consent forms according to the committee procedure.

Histoculture Drug Response Assay

Histoculture drug response assay was done according to the methods reported by Furukawa et al[11] in 1995. In brief, the cancerous portions of the specimens were minced into pieces approximately 10 mg in weight, then placed on prepared collagen surfaces in 24-well microplates. Collagen sponge gels manufactured from pig skin were purchased from Sumitomo Medical Inc, Japan. The plates were incubated for seven days at 37°C, in the presence of drugs dissolved with Roswell Park Memorial Institute 1640 medium containing 20% fetal calf serum, they were then kept in a humidified atmosphere containing 95% air and 5% CO_2. Concentrations of paclitaxel (Bristol-Myers Squibb, New York City, NY) used in this study were 256, 128, 64, 32, 16, 8, 4, and 2 μg/mL. Duplicate samples were used for each concentration assessment.

After histoculture, 100 μ/L Hank balanced salt solution containing 0.1 mg/mL type I collagenase and 100 μ/L 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution, dissolved in 5 mg/mL phosphate buffer solution, were added to each culture, which was then incubated for another 16 hours. Following extraction with dimethyl sulfoxide, absorbance of the solution in each well was read at 540 nm. Absorbance/g of cultured tumor tissue (optical density/weight) was calculated from the mean absorbance of tissue from 2 culture wells, and the tumor-tissue weight was determined prior to culture. Inhibition rate was calculated using the following formula: (1 − mean absorbance of treated tumor/weight/mean absorbance of control tumor/weight) × 100 (%).

Dose–response Curve

The dose–response curve (Figure 1) in each case was constructed by the following formula if the curve was judged to reflect hormesis using the criterion specified below (Statistical Analysis section) by the measured and plotted curve:

$$y = (a - b/(1 + \exp(c \times \log(x) - d))) /(1 + \exp(e \times \log(x) - f)) + g. \quad (1)$$

Inhibition rate and drug concentration were shown as *y*-axis and *x*-axis, respectively.[12,13] Using the coefficients obtained from the equation, the following parameters were calculated: maximal response of hormesis = 100 × b/(a-b), slope factor of hormesis = c, slope factor of cytotoxicity = e, ED_{50} of hormesis = exp(d/c), ED_{50} of cytotoxicity = exp(f/e), reduction threshold (RT) = drug concentration on "y = baseline," maximum stimulation = the highest actual stimulation rate.

Dose–response curve without hormesis was constructed using the following formula:

$$y = a + b/(1 + \exp(c \times \log(x) - d)). \quad (2)$$

Inhibition rate and drug concentration were shown in *y*-axis and *x*-axis, respectively. In the nonhormetic cases, the following parameters were calculated: maximal response = 100 × b/(a + b), ED_{50} = exp(d/c), slope factor = c. Dose–response curve of each specimen was reconstructed using these values.

Statistical Analysis

When the measured values showed a pattern of decrease either initially or after showing a pattern of momentary plateau as the concentration of paclitaxel increased, we judged the dose–response curve to be nonhormetic and applied nonhormetic

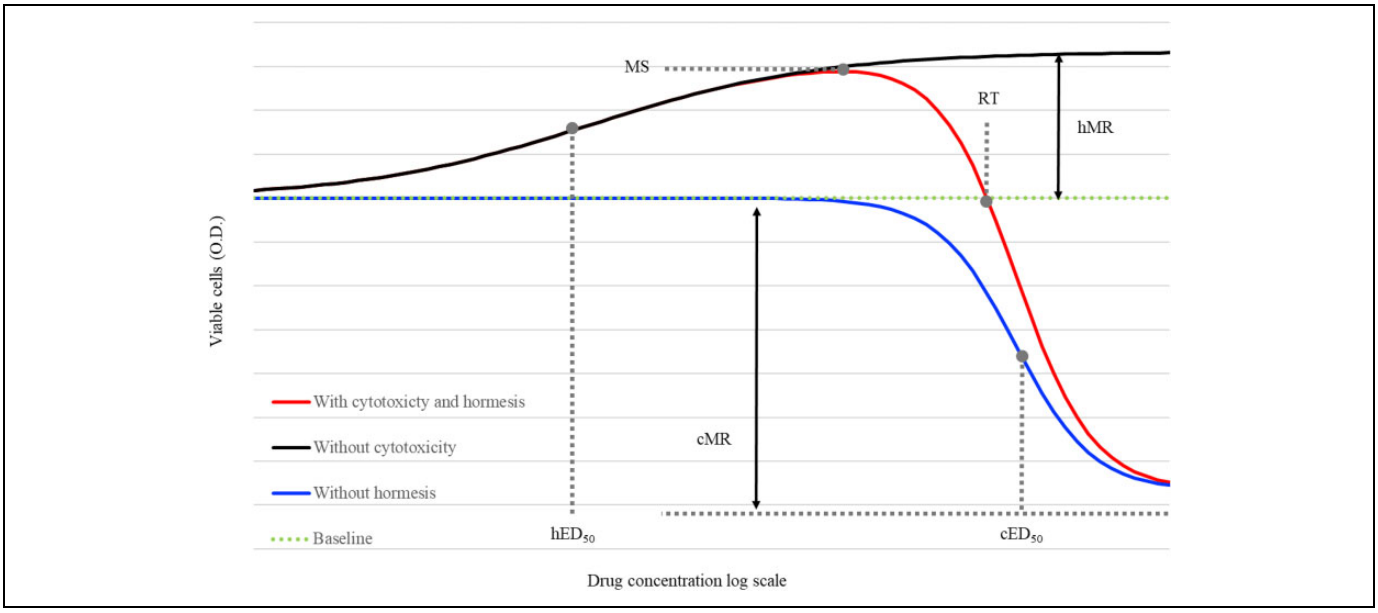

Figure 1. Theoretical dose–response model for anticancer agents with a hormesis. Blue line corresponds to the dose–response curve with cytotoxicity only, black line corresponds to hormesis only, and red line corresponds to cases with hormesis and cytotoxicity. cED_{50} indicates ED_{50} of cytotoxicity; hED_{50}, ED_{50} of hormesis; hMR, maximal response of hormesis; MS, maximum stimulation (the highest stimulation rate); RT, reduction threshold (the drug concentration on y = baseline).

Equation 2. Akaike information criterion (AIC) was calculated[14] in relation to each of the considered fitted dose–response models (hormetic Equation 1 vs nonhormetic Equation 2) when a clear increase of the measured values was observed at a lower concentration of paclitaxel. Eight of the 9 hormetic cases showed small values with a difference of ≥ 2. We included the remaining one case with a difference <2 into the hormetic group because this case could be applicable to either model and we speculated that it might show hormetic response. Each specimen was measured twice. We used all measured values for the AIC calculation and described the data plot of the graph as mean values.

All estimates are reported as mean $\pm$ standard deviation. Student *t* test, Fisher exact test, and analysis of variance were employed to evaluate the significance of differences between groups. A *P* value $<.05$ was considered to indicate a statistically significant difference.

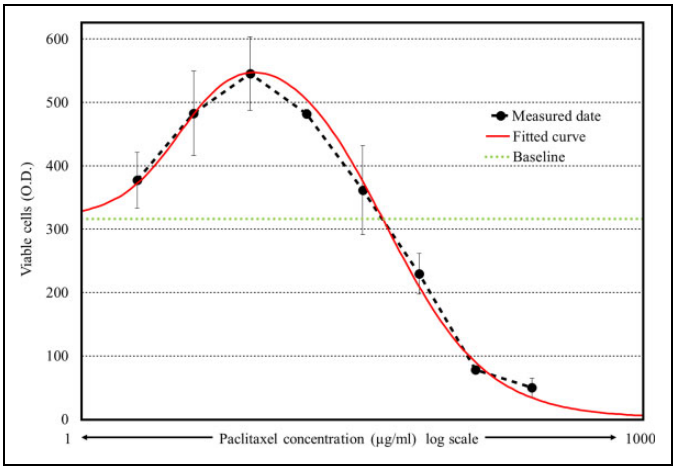

Figure 2. Example of the hormetic dose–response curve of breast cancer specimen for paclitaxel in the histoculture drug response assay. Black markers correspond to the measured absorbance values (OD). Error bars represent mean $\pm$ SD. Red line is the calculated dose–response curve. SD indicates standard deviation; OD, optical density.

Results

An example of the hormetic dose–response curve of breast cancer specimen for paclitaxel in the HDRA is shown in Figure 2. By plotting the measured data (black marker) and fitting it to the formula, dose–response curve is drawn (red line) to show the effect of hormesis.

The dose–response curves of each tumor are shown in Figure 3, the parameters of which are summarized in Table 2. Nine specimens had dose–response curves with hormesis caused by paclitaxel in the HDRA (H group). The remaining 13 specimens did not show hormesis (N group). All tumors in H group were invasive ductal carcinoma, whereas N group contained special types, such as mucinous carcinoma. There was no difference between the 2 groups in nuclear grade, histological grade, estrogen receptor, progesterone receptor, or human epidermal growth factor receptor type 2 status; ED_{50} of anticancer effect is 44.6 $\pm$ 4.2 μg/mL in H group and 26.7 $\pm$ 3.5 μg/mL in N group ($P = .0036$). The mean value of maximum response was 94.6% $\pm$ 2.0% in H group and 90.0% $\pm$ 1.6% in N group ($P = .0923$), and slope factor was 5.8 $\pm$ 1.6 vs 10.5 $\pm$ 1.3, respectively ($P = .0364$; Figure 4).

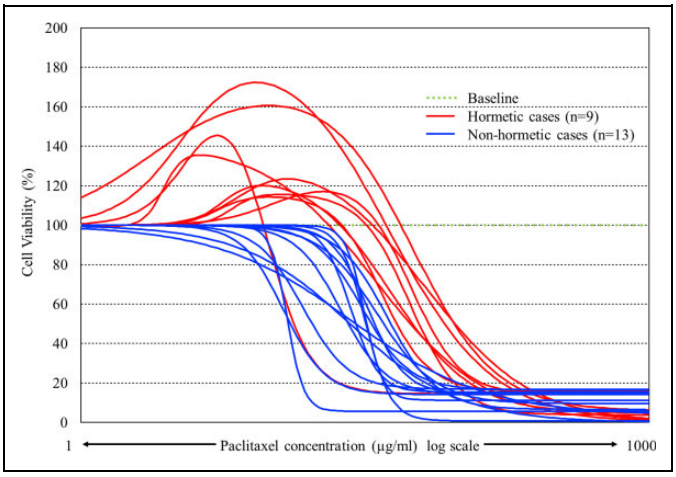

Figure 3. Dose–response curves of paclitaxel in 22 tumors. Hormesis presented in 9 cases (red lines) but not in 13 cases (blue lines).

Table 2. Correlation Between Pathological Factors and Parameters of the Dose–Response Curve.

		Hormetic Cases (n = 9)	Nonhormetic Cases (n = 13)	*P* Value
Histologic type		Invasive ductal carcinoma	Invasive ductal carcinoma	8
			Special type	5
Nuclear grade				1
	1, 2	6	9	
	3	3	4	
ER				1
	Positive	8	12	
	Negative	1	1	
PgR				.6740
	Positive	6	7	
	Negative	3	6	
HER2				1
	Positive	2	4	
	Negative	7	9	
cED_{50}, μg/mL		44.6 ± 4.2	26.7 ± 3.5	.0036
MR, %		94.6 ± 2.0	90.0 ± 1.6	.0923
SF		5.8 ± 1.6	10.5 ± 1.3	.0364

Abbreviations: cED_{50}, ED_{50} of cytotoxicity; ER, estrogen receptor; HER2, human epidermal growth factor receptor type 2; MR, maximal response; PgR, progesterone receptor; SF, slope factor.

Discussion

In 1943, Southam and Ehrlich reported that extracts from red cedar trees enhanced the metabolism of fungi at low concentrations yet inhibited it at higher concentrations.[15] This growth stimulation at low doses and apparent inhibition at higher doses was named "hormesis." In the human body, similar hormesis on normal cells, mainly that caused by radiation therapy, has been reported.[16-18] In addition, more than 120 chemical agents have reportedly shown hormetic-like biphasic dose–responses on over 30 kinds of cancers.[5]

Like antimicrobial susceptibility testing in infectious diseases, chemosensitivity tests, such as succinate dehydrogenase inhibition (SDI) test, collagen gel droplet-embedded test (CD-DST), and HDRA in cancer diseases, have been clinically used for the selection of anticancer agents.[19-21]

Succinate dehydrogenase inhibition test and CD-DST can be done using cancer cells; SDI test has the advantage of it being a simple, low-cost procedure, CD-DST benefits from quasi in vivo chemosensitivity assessment. Histoculture drug response assay requires fresh tissue containing cancer cells and surrounding stromal cells to assess chemosensitivity of several anticancer agents, but it can offer high evaluability through maintenance of cell-to-cell contacts.

We previously reported that, using MTT assay, A549 nonsmall-cell lung cancer (NSCLC) cell line showed a hormetic response to anticancer agents in vitro.[13] Another retrospective study using HDRA data from NSCLC reported an IR less than zero, in other words, cancer cell proliferation with coculture of anticancer agents was found in some cases. Moreover, the frequency of cancer proliferation to anticancer agents ranged from 0.8% of mitomycin to 11.6% of irinotecan, suggesting possible hormesis in NSCLC.[9] In the current study, hormetic dose–response relationship of paclitaxel was measured in surgically resected breast cancer specimens using HDRA. Hormesis was observed in 9 (41%) of the 22 cases. In several cases, chemosensitivity of the breast cancers to cisplatin and gemcitabine was also examined in the same way. Hormesis was observed in 3 of 4 cases (75%) of cisplatin and in 1 of 2 (50%) cases of gemcitabine. These results suggest that the hormesis might clinically occur in patients undergoing chemotherapy, especially in patients undergoing metastatic breast cancer treatment with less intensive chemotherapy.

Hormesis may exist in patients with low ED_{50}, as well as in patients with high ED_{50}. There is a possibility, however, that it cannot be detected by our method because of the measurement range limit on paclitaxel concentration. We propose that, if it does exist, hormesis is not a clinical matter in patients with low ED_{50} because the hormetic concentration is much lower than that of clinical paclitaxel concentration. Although clear cutoff concentration could not yet be set, the ED_{50} of the H group tended to be higher than that of the N group, suggesting possible resistance to paclitaxel through hormesis in some breast cancer specimens with high ED_{50}.

Our study has several limitations. First, the sample size was too small to clarify the factors that affected hormesis. Second, due to the lack of clinical outcome using the same anticancer agent evaluated in this study, we cannot evaluate the degree to which hormesis would affect disease progression in case of tumor relapse. A third limitation is that this study evaluated hormesis of only one anticancer agent, paclitaxel. However, correlation between clinical outcome and HDRA results has reportedly been good. In some cases, hormesis might therefore contribute to disease progression.

In conclusion, hormesis was detected in surgically resected breast cancer specimens using HDRA. Hormesis might be a factor in patients with progressive disease during paclitaxel monotherapy.

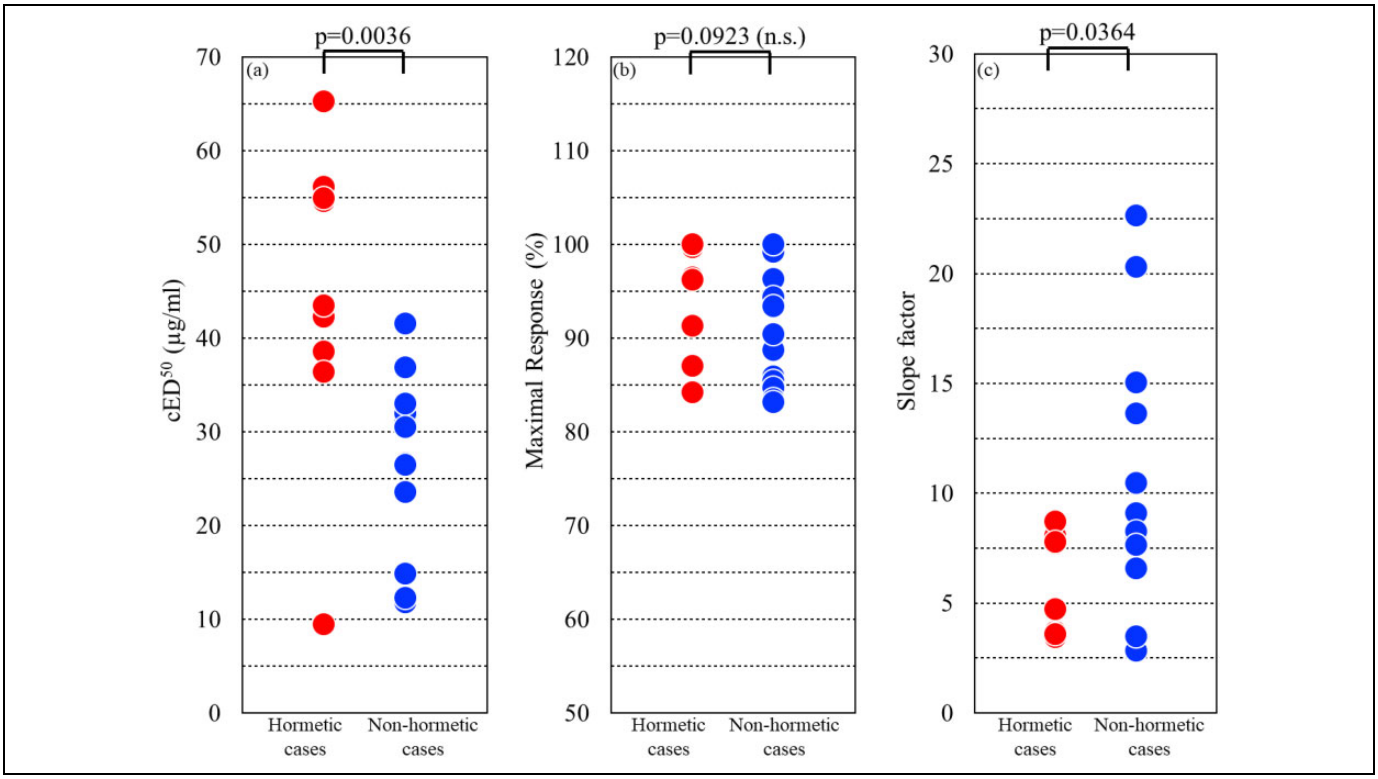

Figure 4. Parameters in relation to hormesis. Dot plots show the values of the parameters and the horizontal bars show the average of each case. cED_{50} indicates ED_{50} of cytotoxicity.

Authors' Note

The study was approved by the Wakayama Medical University Hospital Institutional Review Board (2383). This is retrospective research including the information on HDRA obtained from the previous study. Therefore, we disclosed the study information at onsite at the relevant facilities instead of using a consent form according to committee procedure.

Acknowledgements

We acknowledge proofreading and editing by Benjamin Phillis at the Clinical Study Support Center at Wakayama Medical University.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported in part by a Grant-in-Aid for Scientific Research (C) (15K10268) from Japan Society for the Promotion of Science.

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