Research Article

Comparison of Cantharidin Toxicity in Breast Cancer Cells to Two Common Chemotherapeutics

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As part of a larger study synthesizing a more directed form of chemotherapy, we have begun to assess the efficacy of different potential toxins that could be delivered locally rather than systemically. In doing so, we hope to reduce the systemic side effects commonly observed, while maintaining a high level of toxicity and eliminating the need for metabolic alterations. In a search for this more efficient method for killing cancerous cells, we have begun studying cantharidin, a toxin used in traditional Chinese medicine, as a potential chemotherapeutic. Using an MTT cell viability assay, the toxicity of cantharidin was compared to both cyclophosphamide and paclitaxel in three different breast cancer cell lines: MCF-7, MDA-MB-231, and SK-BR-3. Increasing the concentration of chemotherapy drugs did decrease cell viability in all cell lines when cantharidin and cyclophosphamide were applied; however, differences for paclitaxel were cell-specific. Additionally, cantharidin exhibited the highest decrease in cell viability regardless of cell type, indicating it may be a much more potent and less specific chemotherapeutic. These results will help us move forward in developing a potentially more potent treatment for breast cancer that might eliminate the need for subtype-specific treatments.

1. Introduction

Breast cancer is the second leading cause of cancer related deaths in women [1]. While several targeted chemotherapeutics have been developed, the use of these drugs is limited to those patients who exhibit the proper cellular profile, such as triple negative or HER2 positive [2–7]. In lieu of these targeted therapies, a more nonspecific treatment must be utilized. Cyclophosphamide and paclitaxel are two commonly used chemotherapeutics for breast cancer [8]. Cyclophosphamide is an alkylating agent from a class of nitrogen mustards that will damage DNA, thus preventing cancer cells from reproducing [9, 10]. Paclitaxel interferes with the cell cytoskeleton by altering microtubule rearrangement [11]. Paclitaxel was the first taxane used in clinical trials and found to be used with ovarian and breast cancers resistant to chemotherapy and has been used in vivo studies for inhibiting mammary cancer growth, delaying mammary adenocarcinomas, and decreasing tumor angiogenesis [12]. Usage of paclitaxel with radiation, in initial therapy, was shown to improve survival [13].

Overall, both of those toxins have produced good outcomes; however, when these toxins are used they are injected intravenously and spread throughout the whole body, indiscriminately causing death to both cancerous and noncancerous cells [14–17]. Commonly reported side-effects include hair loss, bladder damage, joint pain, and anemia [18, 19]. Additionally, while these drugs have typically been used for nonspecific treatment of breast cancer, they may in fact show mixed efficacy depending on the genetic background of the tumors, with some tumors now showing paclitaxel resistance [20–22]. It is our long-term goal to identify compounds which might exhibit higher levels of cell death while having reduced specificity and develop a mechanism for a more localized delivery of these compounds to reduce systemic toxicity.

Therefore, we have decided to examine cantharidin, a natural toxin that can induce apoptosis. Cantharidin is
terpenoid that is secreted by several species of beetles of
the Meloidae family, primarily the blister beetles, cardinal
beetles, and soldier beetles, along with the Spanish fly Lytta
vesicatoria [23]. It has been used extensively in traditional
Chinese medicine as a topical treatment for MCV infections
and warts [24]. Recent studies have indicated that cantharidin
can act via inhibition of protein phosphatases I and II A,
activation of p53, inhibition of CDK1, and production of
reactive oxygen species [24–27]. However, as cantharidin is
a potent inducer of apoptosis, its safety as an anticancer agent
is questionable. Li et al. [28] reported cantharidin selectivity
in inducing cell death in pancreatic cancer over normal
pancreatic cell lines, and cantharidin has shown potential for
use in the treatment of colon and liver cancers [29, 30]; it
is also highly toxic to the gastrointestinal tract and kidneys
which can result in mortality [31, 32].

Our long-term goal is to create a chemotherapeutic
treatment that could be utilized locally to kill cancerous cells while
leaving the surrounding tissues and rapidly dividing cells
such as hair follicles and red blood cells intact. As part of
this goal, we seek to identify a compound that exhibits a
more rapid and complete toxicity than the currently available
choices. It is our hypothesis that one of the toxins we are
studying will clearly be more toxic than the other two and
thus will be an ideal choice for moving forward in the
development of new chemotherapy treatments. To compare the
cytotoxicity of these potential chemotherapies, and since
different cell backgrounds may result in different responses to
toxin exposure, we have used three cancer cell models: MCF-
7 cells, MDA-MB-231 cells, and SK-BR-3 cells. We applied
cantharidin, cyclophosphamide, or paclitaxel to these cells for
up to 96 hours and assessed cell viability. The results of these
studies are presented herein.

2. Materials and Methods

2.1. Cell Culture. Three mammary adenocarcinoma cell
lines were utilized: SK-BR-3 cells, which overexpress the
Her2 gene, MDA-MB-231 cells, which are estrogen-receptor
negative, and MCF-7 cells, which express the estrogen
receptor. SK-BR-3 and MDA-MB-231 cells were maintained
in DMEM/F12 media (Life Technologies, Grand Island,
NY) supplemented with antibiotics (50 IU/mL penicillin,
50 μg/mL streptomycin, and 5 μg/mL gentamycin sulfate)
and 10% newborn calf serum (Life Technologies, Grand
Island, NY). MCF-7 cells were maintained in Eagle’s modified
essential medium (HyClone, GE Healthcare Bio-Sciences,
Pittsburgh, PA) containing antibiotics as above and 5% calf
serum (PAA, GE Healthcare Bio-Science Corp., Piscataway,
NJ). All cells were maintained at 37 °C in a 5% CO2 humidified
environment.

2.2. MTT Assay. When cells reached a minimum of 75% confluency, they were washed with HBSS, trypsinized, resuspended in media, and plated into one 96-well plate per original 75 cm2 flask. After twenty-four hours, a set of plated cells was treated in triplicate in the respective media containing either 1 to 100 μM cantharidin, 0.1 to 10 μM paclitaxel, or DMSO vehicle control, or 10 to 1000 μM cyclophosphamide
or H2O vehicle control. This treatment was repeated on fresh
cells after 24, 48, and 72 hours.

After a maximum of 96 hours of treatment, an MTT
(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium
bromide, Sigma, St. Louis, MO) based cell viability assay
was performed in the manner of Sargent and Taylor [33].
Briefly, MTT was added to each well to a final concentration
of 0.5 mM, and cells were incubated at 37 °C in a 5% CO2
incubator. After 3 hours, the MTT solution was removed and
cells were solubilized in 200 μL of DMSO. The amount of
stain was quantified using a microplate reader at 570 nM.
The data recorded represented the amount of viable cells in a well
and were normalized to the untreated control wells for each
every experiment. A minimum of four experimental replicates
were performed. Univariate analysis of variance (ANOVA)
was performed using SPSS (IBM SPSS Statistics 21, IBM
Corp., Armonk, NY).

3. Results and Discussion

MDA-MB-231, SK-BR-3, and MCF-7 breast cancer cells were
exposed to a single chemotherapeutic drug at a range of
concentrations spanning the IC50 for up to 96 hours. Viable
cells were stained with MTT dye, which was converted to
formazan by mitochondria in live cells [33]. The amount of
formazan was then quantified using a microplate reader.
Results were compared to the appropriate control, either
dMSO or H2O. Statistical analysis of the resulting data
was performed using SPSS. Univariate ANOVA indicated
cross-interactions between all multifactorial combinations of
cell type, toxin, concentration, and time of exposure, and
thus smaller interactions (toxin, concentration, and time of
exposure plus multifactorial combinations) were examined
within each cell type when appropriate.

Cyclophosphamide is commonly utilized for the treat-
ment of several cancers and is most potent after conversion
to its active form by the liver [34]. We selected a treatment range
of 10 to 1000 μM or a water vehicle control, as these levels
overlap or exceed the reported IC50 range of 0.2–17 μM [35,
36]. Cells that were treated with cyclophosphamide showed
minimal cell death (Figure 1). No significant differences were
observed with up to 1000 μM cyclophosphamide in the
MDA-MB-231 cells (Figure 1(a)). In contrast, at this highest
concentration, both the SK-BR-3 and the MCF-7 cells showed
a reduction in cell viability when compared to the water
control. In the SK-BR-3 cells, only 30% death was observed
consistently at the highest concentration (Figure 1(b)). A
similar difference was only observed at the longest treatment
in the MCF-7 cells (Figure 1(c)). Thus, the MCF-7 and MDA-
MB-231 cells were more resistant to cyclophosphamide than
the SK-BR-3 cells. This was somewhat expected with our
direct application of the toxin, as cyclophosphamide is not
as toxic without being metabolized by the liver [37]. Franke
et al. [38] showed that apoptosis nearly doubled in MCF-
7 cells treated with between 0.001 and 1 μM compared to
control-treated cells, yet little effect was observed in MDA-
MB-231 cells. Even with much higher concentrations of
Cyclophosphamide, we failed to detect similar levels of cell death.

Paclitaxel (Taxol) is another chemotherapeutic which is widely used in breast cancer treatments [39–41]. Paclitaxel has shown mixed efficacy dependent upon cell type as well, with an IC\textsubscript{50} range of 0.02–100 nM [42–45]. Thus, we felt that it was more appropriate to aim for the higher end of this range to ensure cell death in our three cell lines. However, even at this higher dosage, paclitaxel showed limited toxicity (Figure 2). No significant differences in viability were observed at the 24- or 48-hour time points in any of the three cell lines, regardless of concentration used. In MDA-MB-231 cells, we did observe minor reductions in viability at 72 and 96 hours, although this was seen around the middle concentration of 1 \( \mu \)M (Figure 2(a)). In the SK-BR-3 cells, recovery was observed after a temporary decrease in viability at 72 hours (Figure 2(b)). In the MCF-7 cells, a significant effect of time was observed as the 96-hour treatment showed lower survival than the vehicle control for both of the lower concentrations. Recent work has indicated that some cell types may be resistant to paclitaxel due to downregulation of LZTS1 [45, 46]. With the minute toxicological response we detected, combined with the resistance to paclitaxel now being observed, paclitaxel does not appear to be a good selection for a local chemotherapeutic.

Cantharidin has been sought out previously as a potential chemotherapy drug, as it shows a high level of toxicity in a variety of cancerous cell lines [24, 26, 27, 47, 48]. However, limited direct comparisons to current chemotherapeutics have been made. Chang et al. [49] report an IC\textsubscript{50} of 50 \( \mu \)M for cantharidin in MCF-7 cells, thus we selected a range of 1 to 100 \( \mu \)M for our study. Unlike paclitaxel and cyclophosphamide, our data show that cantharidin was able to significantly reduce viability in all three cell lines (Figure 3). At 1 \( \mu \)M cantharidin, up to thirty percent cell death was observed in the MDA-MB-231 cells (Figure 3(a)), and

![Viability of breast cancer cells after exposure to 10 \( \mu \)M, 100 \( \mu \)M, or 1000 \( \mu \)M cyclophosphamide. MDA-MB-231 (a), SK-BR-3 (b), or MCF-7 (C) cells were treated for up to 96 hours with either cyclophosphamide or a water control. Significant differences compared to the vehicle control (\( ^* P < 0.05 \)) are indicated.](image-url)
Figure 2: Viability of breast cancer cells after exposure to 0.1 μM, 1 μM, or 10 μM paclitaxel. MDA-MB-231 (a), SK-BR-3 (b), or MCF-7 (c) cells were treated for up to 96 hours with either paclitaxel or DMSO vehicle control. Significant differences compared to the vehicle control (* P < 0.05) are indicated.

There was more than fifty percent cell death after at least 72 hours of treatment in the higher concentrations. In the other two cell lines, statistically significant cell death compared to the vehicle control was not consistently observed until the 100 μM concentration. However, this is significantly more cell death than was observed for the other two toxins in each of the cell lines examined; when concentration is not factored in, there is a statistically more death for all three cell lines for cantharidin than either cyclophosphamide or paclitaxel (P < 0.001).

While cyclophosphamide and paclitaxel show limited toxicity at their reported IC\textsubscript{50} levels, our data indicate that cantharidin can reduce viability significantly regardless of cell milieu. Thus, cantharidin holds potential as a better chemotherapeutic option for cancers with a variety of genetic backgrounds. As cantharidin is effective without being metabolized by the liver, it could be injected locally into the tumor rather than being dosed systematically. However, we must consider that even with localized dosage, there is still a systemic risk of toxicity. Conflicting reports of cantharidin toxicity to the gastrointestinal and renal systems make the choice to utilize cantharidin a difficult one. There are studies that indicate that, in both \textit{in vitro} environment using pancreatic cells and several clinical trials, cantharidin is more toxic to cancerous cells than noncancerous [28, 50, 51]. However, historical evidence dictates that the kidney and liver have shown particular sensitivity to cantharidin, due to their physiological role in clearance [31, 32, 52]. To reduce the potential toxicity of cantharidin to noncancerous cells, our next goal is to collaborate with chemists at our institution to develop a drug delivery mechanism which will protect the normal tissue and allow for directed cell death only in the area of the tumor. Nanoparticles hold much promise as an encapsulating agent [53, 54]. Additionally, nanoparticles reduce the severity of many of the side effects of conventional chemotherapy [55, 56]. Thus, future studies will focus upon creating a nontoxic shell from liposomes and hollow gold nanoparticles that will encapsulate the...
cantharidin to reduce its toxicity should it come in contact with nontumor cells. Once synthesized, a release mechanism will be identified to ensure delivery of the toxin to only the tumor. Understanding the interaction of these nanoparticles with cancerous and noncancerous cells will allow improved diagnosis and treatment in cancer research, including the delivery of chemotherapy and targeting of tumors.

4. Conclusion

In each of our cell types examined, MDA-MB-231, SK-BR-3, and MCF-7 cells, we observed a high decrease in cell viability after cantharidin was applied when comparing it to the untreated control, reaching more than 80% cell death for each of the cell types after 96 hours of treatment with 100 μM cantharidin. This is substantially more death than was observed for either paclitaxel or cyclophosphamide, two commonly prescribed chemotherapy agents. Thus, we have identified a more potent toxin that may be utilized for local, rather than systemic, dosing.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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