Electro-Turmeric-Therapy for Aggressive Triple Negative Breast Cancer

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Cancer is rising!
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Higher Prevalence in High Income Countries

Share of population with cancer, 1990
Share of total population with any form of cancer, measured as the age-standardized percentage. This share has been age-standardized assuming a constant age structure to compare prevalence between countries and through time.

Source: IHME, Global Burden of Disease

Increased Cancer risk due to Lifestyle factors: smoking, alcohol, diet, and obesity
Current standard of cure is inadequate, a critical need for alternate therapies
Breast Cancer

Worldwide, breast cancer is the most common cancer that women may face in their lifetime (except for skin cancer).

Worldwide, every 19 seconds one woman is diagnosed with breast cancer and every minute one woman loses her life.

In the U.S., every 13 minutes six women are diagnosed with breast cancer and one loses her life.

1 in 8 women in the U.S. are on lifetime risk of developing breast cancer.
Triple Negative Breast Cancer

Difficult to Treat, Aggressive, and Metastatic Phenotype

Worldwide, 20% of breast cancer is Triple Negative Breast Cancer (TNBC)

TNBC lacks estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2/neu) targeted by chemo drugs

Receptor Positive Breast Cancer

- Hormone-receptor binding blocked
  - Cell stops growing
  - Receptor positive cells may stop growing with Chemotherapy treatment

66% 5-year survival rate

Triple negative Breast Cancer

- TNBC Cell
  - Continued growth
  - Triple negative breast cancer cells have none of the 3 important hormone receptors on its surface
  - Triple Negative Breast Cancer cells may NOT stop growing with chemotherapy treatment

× No effective targeted therapy against TNBC

http://triplesteptowardthecure.org/understanding.php
Why Turmeric?

Turmeric: A herb, used in medicine and cooking in India, China, and other Asian countries over 5000 years

Useful to treat Over 150 Diseases Including cancers

Curcumin

Liver diseases
- Alcohol-induced liver disease
- Cirrhosis
- Jaundice
- Fibrosis

Lung diseases
- Hyaline membrane disease
- Bronchitis
- Cystic fibrosis
- Wound healing

Skin diseases
- Eczema
- Psoriasis
- Scleroderma
- Parastic skin diseases

Skeletal or bone anomalies
- Fanconi anemia
- Osteoporosis
- Atherosclerosis
- Hypolipidemia
- Myocardial infarction
- Hypothyroidism

Diabetes
- Heart Disease
- Endocrine disorder

Infectious diseases
- Tuberculosis
- Chicken pox
- Smallpox
- Malaria
- Measles
- Leishmaniasis
- Chronic diarrhea

Other inflammatory diseases
- Inflammatory bowel disease
- UCI

Neurodegenerative disorder
- Parkinson's disease
- Alzheimer's disease
- Lewy body disease

Allergy
- Gout
- Allergic colitis

Anticancerous

Antiseptic

Antifungal

Antioxidant

Turmeric

Why Turmeric?

✓ A natural alternative
✓ No side effects
✓ Cost effective

Exorbitant Cost of Chemotherapy

<table>
<thead>
<tr>
<th>Drug</th>
<th>Disease</th>
<th>Time</th>
<th>Amount needed</th>
<th>Cost</th>
<th>Total Cost</th>
<th>Cost per QALY*</th>
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<tbody>
<tr>
<td>Avastin</td>
<td>NSCLC</td>
<td>5 cycles</td>
<td>4500 mg</td>
<td>$6^{70}/mg</td>
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<td>Tarceva</td>
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<td>Erbitux</td>
<td>NSCLC</td>
<td>18 weeks</td>
<td>6975 mg</td>
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<tr>
<td>Erbitux</td>
<td>CRC</td>
<td>18 weeks</td>
<td>6975 mg</td>
<td>$5^{76}/mg</td>
<td>$40,176</td>
<td>$283,595</td>
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</tbody>
</table>

*QALY: Quality-Adjusted Life Years

Cost of Turmeric: $0.025/g

Why Electrical Pulse?

Local application of electrical pulses renders the cell membrane permeable to impermeable or less permeable anti-cancer drugs, facilitating a potent localized cytotoxic effect.

The Electroporation process: (a) molecules surround the cell after injection; (b) electrical pulses cause pore formation, molecules overcome the membrane; (c) cell membrane reseals, molecules are entrapped; (d) cellular response to the molecules.

Electrical pulses + Turmeric can be a novel alternative for treating TNBC.
Materials and Methods

The Cells:
- MDA-MB-231 - Basal type human adenocarcinoma epithelial TNBC cell line
- Aggressive/metastatic

The Drug-Turmeric Treatment:
- Turmeric stock solution of 100mg/mL was prepared in dimethyl sulfoxide (DMSO)
- 100mg/mL stock solution was added into media containing cells to make 2mg/mL treatment concentration
Materials and Methods

Protocol of Electrical Pulse Application:

• 8 square wave unipolar pulses of 1 Hz frequency at 1200V/cm with 100μs pulse duration used
• BTX ECM 830 Electroporator-square wave-unipolar
• Turmeric treated cells at $1 \times 10^6$ cells/mL concentration were used for electroporation
Materials and Methods

The Experimental Workflow:

MDA-MB-231 cells

- Control
- Electrical Pulse only
- Turmeric (2mg/mL)
- Curcumin (50μM)

  - Electrical Pulse Application
  - Electrical Pulse Application

Cell Viability Assay
Materials and Methods

Statistics:

Hypothesis -

Null \( (H_0) \): \( \mu_1 = \mu_2 = \mu_3 \ldots = \mu_n \)

Alternate \( (H_1) \): Not all means are equal

where, \( n \) = number of independent treatment groups (4)

Procedure for statistics
Results and Discussion

Immediate Cell Viability:

Trypan Blue Assay using Hemocytometer and Microscope

ANOVA Test Results

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<thead>
<tr>
<th>Source</th>
<th>Degree of Freedom (DF)</th>
<th>F Ratio</th>
<th>Prob &gt; F</th>
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The viability of MDA-MB-231 cells immediately after different treatments

* Treatments not connected by the same letter are significantly different from each other (p < 0.05).
Results and Discussion

Immediate Cell Viability:

Trypan Blue Assay using Nexcelom Bioscience Cellometer

ANOVA Test Results

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The viability of MDA-MB-231 cells immediately after different treatments

Treatments not connected by the same letter are significantly different from each other (p < 0.05).

https://www.photonics.com/pr34000/Automated_cell_counter
Results and Discussion

Cell Viability at 24h after treatment:

MT Viability Assay (Promega)

ANOVA Test Results

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The viability of MDA-MB-231 cells at 24h for different treatments

* Treatments not connected by the same letter are significantly different from each other (p < 0.05).
Conclusions

• Electro-turmeric therapy is designed as an effective method to increase therapeutic efficacy and reduce side effects, if any.

• The turmeric + eight 1200V/cm, 100μs pulses are effective in reducing the viability to 4.75%, which is statistically not different from the 3.3%, obtained for turmeric only.

• The use of electrical pulses is preferable due to the multimodality treatment that could be obtained due to synergy of electrical pulses and turmeric.

• This therapy is cheap and effective and has the potential to treat the unmet need for aggressive TNBC.
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References


Purdue University
Thank you

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