Ellagic acid induces apoptosis in neuroblastoma via multiple signaling pathways

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Introduction

❑ Background

- Neuroblastoma is the most common extra cranial solid tumor and aggressive type of pediatric cancer and has its roots in immature nerve cells, predominantly impacting children under the age of five [1].
- Neuroblastoma can develop in various Body locations, most commonly in the adrenal glands above the kidney but also in the spine, chest, abdomen, or pelvis.

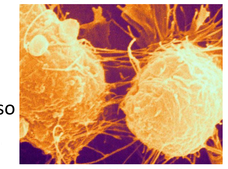


Fig 1:Neuroblastoma Cell [8]

❑ Signs and Symptoms of Neuroblastoma

- Vary depending on tumor location, size, and metastasis, and not all children with neuroblastoma experience all symptoms.
- Abdominal swelling, change in bowel habits, Weight loss, Fatigue, Fever, Bone pain, Proptosis (eye bulging), Bruising or bleeding[1].

❑ The role of NMYC in neuroblastoma

N-Myc, encoded by the MYCN gene, is a pivotal transcription factor in neuroblastoma cells. Its amplification is closely associated with an aggressive form of the disease, making it a significant prognostic marker. Specifically, MYCN amplification leads to rapid tumor cell proliferation and prevents the differentiation of neuroblastoma cells into a less aggressive phenotype[2, 3].

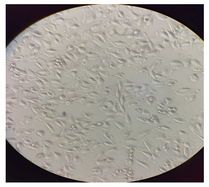


Fig 2:NMYC neuroblastoma cells

❑ Treatment Modalities for Neuroblastoma

➢ Treatment strategies for neuroblastoma include surgery, chemotherapy, radiation therapy, immunotherapy, chemotherapy Doxorubicin.

➢ Mechanism of Action of Doxorubicin

- Intercalation into DNA: Doxorubicin inserts itself between DNA base pairs.
- Disturbs the DNA structure, preventing DNA replication and transcription.
- Inhibition of Topoisomerase II: Doxorubicin binds to topoisomerase II, an enzyme that relaxes supercoiled DNA. This binding prevents the re-ligation of DNA strands, leading to DNA breaks.
- Generation of Free Radicals: Doxorubicin can undergo redox reactions, producing reactive oxygen species (ROS)
- Effect: Combined actions lead to cell cycle arrest and apoptosis in cancer cells[4].

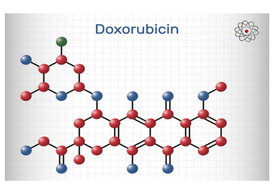


Fig 3: Chemical structure of Doxorubicin [9]

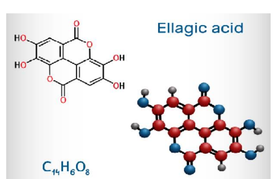


Fig 4: Chemical structure of Ellagic acid [10]

➢ Ellagic Acid and Its Potential Role in Cell Death

- Ellagic acid, a natural polyphenolic compound in fruits and vegetables, is of significant interest due to its potential health benefits, such as antioxidative, anti-inflammatory, and anticancer properties.
- Multiple studies have explored its ability to hinder cancer cell growth and proliferation.
- Ellagic acid is thought to exert its anticancer effects through pathways like inducing apoptosis, inhibiting cell proliferation, anti-angiogenic effects, and inhibiting DNA repair in cancer cells[5, 6].

❑ Aim of study

- The aim of this research was to investigate the anti-cancer properties of ellagic acid on NB cells, with a focus on its potential to modulate various signaling pathways, notably those related to proliferation and apoptosis in neuroblastoma.

METHODS

❑ Cell Culture and Treatment

- Utilized neuroblastoma MYCN cells
- Seeded cells at a density of 10,000 cells/mL (1,000 cells per well) in 96-well plates
- Allowed cells to adhere and grow for 12 hours at 37°C, 5% CO2, and 95% humidity
- Treated cells with Ellagic acid at concentrations ranging from 50 µM to 0.003 µM
- Treated cells with Doxorubicin across a range of 30 µM to 0.003 µM for 48 hours
- Fixed treated cells with 10% trichloroacetic acid (TCA)
- Post-washing, stained cells with Sulforhodamine B (SRB)
- Determined cell viability using a plate reader by assessing the absorbance of the SRB stain which is indicative of cell biomass[5]
- Each treatment was administered three times individually

Fig-5: Cell Culture in Microfluidic Droplets [11]

❑ Statistical Analysis

- Data from the SRB assay were analyzed to determine cell growth and treatment effects.
- Results are presented as the mean ± standard deviation of at least three replicates.
- Statistical significance was determined using a two-way analysis of variance (Student's t-test), with $p < 0.05$ considered statistically significant.
- This method section describes the specific procedures followed in the experiment, including cell culture, treatment, and the SRB assay used to assess cell viability and treatment efficacy[7].

RESULTS

❑ IC50 Determination of Ellagic Acid on MYCN-2 Tet-on Cells

➢ **Experimental Replication**:

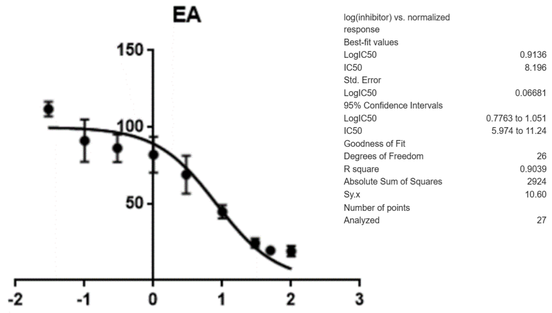
- All plates are prepared and processed **in triplicate** to ensure data accuracy and reproducibility.

➢ **Data Presentation**:

- **X-axis**: Concentration of drug (Log µM).
- **Y-axis**: Cell viability.

➢ **Results**:

- For Ellagic acid treatment alone:
  - **IC50 Value**: 8 µM
  - Indicates the concentration at which Ellagic acid inhibits 50% of cell growth.



log(inhibitor) vs. normalized response	
Best-fit values	
LogIC50	0.9136
IC50	8.196
Std. Error	
LogIC50	0.06681
95% Confidence Intervals	
LogIC50	0.7763 to 1.051
IC50	5.974 to 11.24
Goodness of Fit	
Degrees of Freedom	26
R square	0.9039
Absolute Sum of Squares	2924
Sy.x	10.60
Number of points	
Analyzed	27

Fig 6: Dose-response curve of Ellagic acid

❑ IC50 Determination of Doxorubicin on MYCN-2 Tet-on Cells

➢ **Experimental Design**:

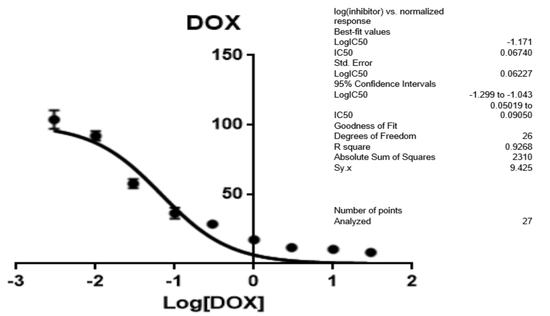
- All plates are prepared and processed **in triplicate** to ensure data consistency and reliability.

➢ **Graphical Presentation**:

- **X-axis**: Concentration of Doxorubicin (Log µM).
- **Y-axis**: Cell viability.

➢ **Preliminary Results**:

- For Doxorubicin treatment alone:
  - **IC50 Value**: 0.07 µM
  - Represents the concentration at which Doxorubicin inhibits 50% of cell growth.



log(inhibitor) vs. normalized response	
Best-fit values	
LogIC50	-1.171
IC50	0.06740
Std. Error	
LogIC50	0.06227
95% Confidence Intervals	
LogIC50	-1.299 to -1.043
IC50	0.05019 to 0.09050
Goodness of Fit	
Degrees of Freedom	26
R square	0.9268
Absolute Sum of Squares	2310
Sy.x	9.425
Number of points	
Analyzed	27

Fig 7: Dose-response curve of Doxorubicin

Discussion

- Investigating novel drugs and alternative treatments for relapsed high-risk neuroblastoma (NB) patients is crucial.
- Treating these patients remains challenging due to aggressive tumor phenotypes and complex mechanisms that promote resistance to treatments and recurrence[1].
- This study focuses on examining the potential of ellagic acid as a treatment for NB.
- Previous research has shown surprising anticancer effects of ellagic acid in various malignancies[5, 6].
- Our results demonstrate that ellagic acid significantly reduced NB cell numbers compared to untreated controls.
- According to the IC50 of both drugs ellagic acid, a naturally occurring polyphenolic compound, exhibits potent anti-cancer properties when tested on NB cells.

Conclusion

- Our study, the first of its kind, investigates the efficacy of ellagic acid on NB. The findings reveal that ellagic acid exerts multiple effects on NB cells, ultimately reducing their viability and inducing cell death in a time- and dose-dependent manner compared to doxorubicin. Given its natural origin, ellagic acid shows promise as a potential treatment for NB, with the potential to reduce the side effects associated with chemotherapeutic agents and enhance cell death within cancerous tissues.
- The next phase of our research will delve into the mechanisms by which ellagic acid impacts various signaling pathways, including those related to apoptosis and anti-proliferation in cancer cells.

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