

Arresting the Uncontrolled Growth of Pancreatic Cancer Cells using Electrical Pulses and Low Dose Cytotoxic Agent

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Abstract—Cancer is an electrostatic phenomenon. When the transmembrane potential (difference in charge within the cell and outside the cell membrane) is about -65mV , the cell is in resting phase. However, when it becomes -15mV , the cells are in constant proliferation. With the application of electrical pulses of appropriate magnitude and frequency, it has been proven, that it is possible to arrest the cell and hence the tumor growth. Thus electrotherapy is ideal, especially for advanced chemo-resistant tumors, such as pancreatic carcinoma. For these terminal patients, left with no traditional alternatives, electrochemotherapy is a boon. With hardly 10% survival rates, advanced pancreatic tumor patients need an attractive alternative, that is efficient and economical and towards this, electrochemotherapy is useful, as most of the pancreatic cancers are chemoresistant, as studied by MD Anderson Cancer Center with 21 pancreatic cell lines. They need an additional agent to open up the cells to uptake the cells. Towards this, it is found that using electrical pulses of high and low intensities along with durations of short and long, it is possible to arrest the cell growth. To study this, we used Panc-28, human pancreatic cancer cells along with Gemcitabine drug, an anticancer agent, commonly administered for pancreatic cancer. Eight pulses of 1200V/cm , $100\mu\text{s}$ duration reduced the viability to 20% compared to 100% of the control (untreated sample) and two pulses of 500V/cm , 25ms reduced the viability to still lower, to 14%. $50\mu\text{L}$ of $100\mu\text{M}$ Gemcitabine was used for this purpose. With drug only the cell viability was as high as about 60% with both 50 and $25\mu\text{L}$ volume of the drug at the $100\mu\text{M}$ concentration. These results illustrate that using a low dose of cytotoxic agent along with electrical pulses, it is possible to arrest the uncontrolled growth of pancreatic adenocarcinoma tumors. Due to its simplicity and physical nature that is applicable to all histologies of cancers, it could be easily transferred for clinical practice.

INTRODUCTION

With 46,420 new cases and 39,590 deaths (85%) in 2014, pancreatic cancer is the most deadly cancer. It is the 4th leading cause of cancer deaths in both men and women [1], although the incidence rate is 9th for women and 10th for men. It has a five year survival rate of 6% and one year survival rate of 27%. Fig. 1 shows the survival rate of pancreatic cancer [2]. This poor survival rate could be attributed to the location and proximity of pancreas. Fig. 2 shows where the pancreas is located and how it is surrounded by many

important organs, such as stomach, liver, spleen and blood vessels [3]. It is not only difficult to identify its tumor at early stages, once it is diagnosed; it is not easy to treat it too [4, 5].

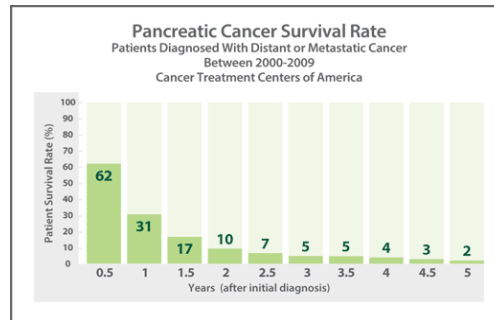


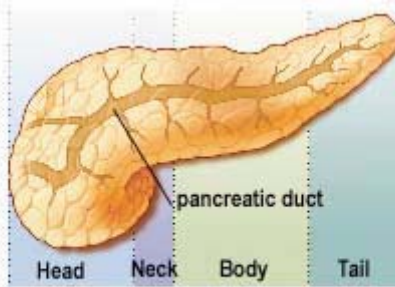
Fig. 1. Poor pancreatic cancer Survival Rate [2].



Fig. 2. Location of Pancreas [3].

Pancreas is 6 inches long, and is an organ of the digestive system, located deep in the abdomen behind the stomach [4]. It is shaped like a pear, sitting on its side. Fig. 3 shows a pancreatic structure [6]. The wide end, known as head, lies in the right middle of the abdomen, tucked into the first part of small bowel (the duodenum). The middle sections, called as neck and body are behind the stomach. The tail, thin end is located on the left of abdomen, next to spleen. It has the large main pancreatic duct and common bile duct. In addition, there are two important blood vessels associated with the pancreas, the superior mesenteric artery and the superior mesenteric vein, found in the neck of the pancreas.

Only up to 20% of pancreatic tumors are removed by surgery. The Whipple procedure, which is done most commonly, involves the removal of the head of the pancreas, lymph nodes draining that area of the pancreas, part of bile duct, gallbladder, and most of first part of the small bowel [4]. A classic Whipple involves the removal of the whole duodenum and part of stomach, in addition to the above. Total pancreatectomy involves the whole pancreas and the spleen, in addition to the rest of the parts as in Whipple.



Pancreas sections used in defining tumor(s) location.

Fig. 3. Pancreatic Parts-Head, Neck, Body, and Tail [6].

All these involve not only significant patient discomfort and after life, but also problems in eating and normal wellbeing. With the removal of pancreas, the patient has to take diabetic treatment for the rest of the life as well as take medication for compensating the loss of pancreatic enzymes due to loss of pancreas [4].

Both stages III and IV cancers and metastatic tumors cannot be removed by surgery.

The radiation and chemotherapy have their own side effects. Especially, pancreatic cells are known for their chemoresistance. It is indicated in a MD Anderson Cancer Center study that 21 pancreatic cell lines are resistant to many of the chemo drugs, including Gemcitabine, the classical drug approved in 1996 for first line treatment of pancreatic cancers. The physical and emotional suffering of the patients with pancreatic cancer cannot be overstated. All these indicate the need for alternate therapy, especially for inoperable, chemo and radio resistant tumors. Towards this, we studied the application of electrical pulses for loco-regional delivery of low dose gemcitabine for an efficient, effective, and economical treatment of pancreatic cancers, that can be transferred to clinical practice.

It is predicted that by 2030, it will double in number and 2.4x (240%) in death rates. This indicates that conventional surgery, chemo and radio therapies used for this cancer are inadequate and there is an urgent and critical need for alternate therapies. Towards this we explore the application of electrochemotherapy (ECT) to arrest the uncontrolled growth of pancreatic cancer cells. This two-prong attack using both a chemodrug and electrical pulses exploits the synergy of these both complementing techniques, enabling low-dose usage with electrical pulses enhancing the cellular plasma membrane permeability, which are typically poorly or impermeable to external molecules, such as chemodrugs.

The success of ECT is demonstrated in various clinical applications [7-10]. Eight, 1200V/cm, 100us pulses are used along with bleomycin chemodrug. In our study, we explored the use of gemcitabine chemodrug, used as a first line of treatment, along with electrical pulses. For this purpose Panc-28, human pancreatic cells were used.

MATERIALS & METHODS

A. The Cell Line

MDAPanc-28, human pancreatic ductal epithelial carcinoma cells (obtained from NCCS,

Pune, India) were cultured in DMEM HG (DMEM High Glucose) supplemented with 10% fetal bovine serum (FBS) at 37° C in a humidified 5% CO₂ incubator. This cell line was derived from the tissue of a 69 year old woman with locally advanced adenocarcinoma tumor of the body (Fig. 3) of the pancreas at the MD Anderson cancer Center. It had poorly differentiated adenocarcinoma, with poor prognosis and the patient dies after four months, having chemotherapy, radiation, laparotomy surgery and intraoperative radiation. The cells were plated at the densities of 1×10⁶. The cells were trypsinised with trypsin and centrifuged at 1100 rpm for five minutes. The supernatant was discarded and fresh medium was added to pellet and made it into suspension. Fig. 4 shows a cell morphology of the Panc-28 cells [11].

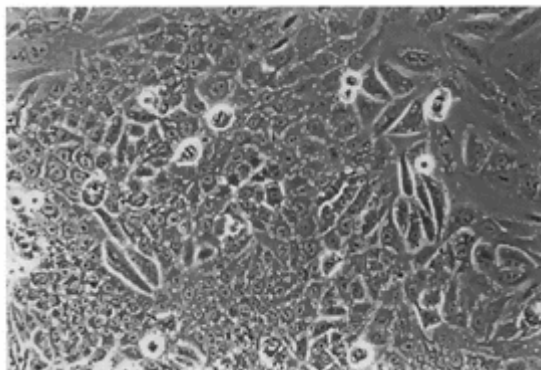


Fig. 4. MDA Panc-28 Cells [11]

B. The Drug

Gemcitabine is an antimetabolite chemo agent that is used as first line drug for systemic chemotherapy of pancreatic cancer. Fig. 5 shows its structure. Its chemical formula is C₉H₁₁F₂N₃O₄•HCl. It replaces one of the building blocks of nucleic acids, the cytidine, during DNA replication. This process arrests tumor growth, as new nucleosides cannot be attached to the faulty nucleoside, resulting in apoptosis. Gemcitabine is used also in non-small cell lung cancer, bladder cancer, ovarian cancer and breast cancer.

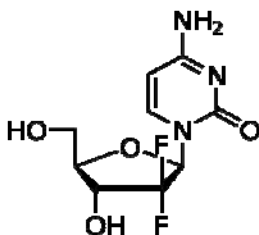


Fig. 5. The structural formula of Gemcitabine [12].

Some of the more common side-effects include bleeding gums, blood in urine or stools, burning, crawling, itching, numbness, tingling feelings, diarrhea, dizziness, fever or chills, joint pain, lack of appetite, lack of strength, muscle aching, nausea and nose bleeds. Its

response rate is a poor 25%. Hence using electrochemotherapy is an ideal way to enhance its uptake to affect the killing of proliferating cells.

We purchased Gemcitabine (GEMIBINE 1000) from the Intas pharmaceuticals, Ahmedabad, India. A concentration of $100\mu\text{M}$ was used in this study.

C. The Electroporation Technique

Cells were dissociated from flask by treatment with 0.25% trypsin/EDTA solution (Invitrogen, Carlsbad, CA). Cells were counted using a cello meter and resuspended in MEM media to a concentration of 250,000 cells per 0.4cm cuvette tube. A BTX 830 square wave electroporator (Genetronics, San Diego, CA) along with 0.4cm cuvettes were used for electroporation. Various pulse conditions were applied at one-second interval.

D. The Viability Assay

To study the effectiveness of the electroporation along with gemcitabine, viability assay was performed, immediately after electroporation. For this purpose, $10\mu\text{L}$ of cells were counted using a hemocytometer.

RESULTS AND ANALYSIS

Fig. 6 shows the viability for various conditions. The control with no treatment is considered as 100% viability. The drug only sample has 60% viability, indicating the drug resistance of these cells to the drug, gemcitabine. When treated with eight, $500\text{V}/\text{cm}$ pulses at various pulse durations, the viability reduced significantly, indicating cell kill, the desired effect to arrest the uncontrolled growth of the pancreatic tumors. With 1ms pulses, the viability reduced to 45.9% and it decreased to 28% at 10ms and drastically reduced to 18% at both 20 and 25ms pulses. The drug concentration was the same in all cases ($50\mu\text{L}$ at $100\mu\text{M}$). This indicates that we can obtain the desired cell death for a given drug dose. The saturation effect of the pulse duration is seen when there is not much difference between 20 and 25ms pulse durations. The sensitivity is lost when the pulse conditions are close to each other.

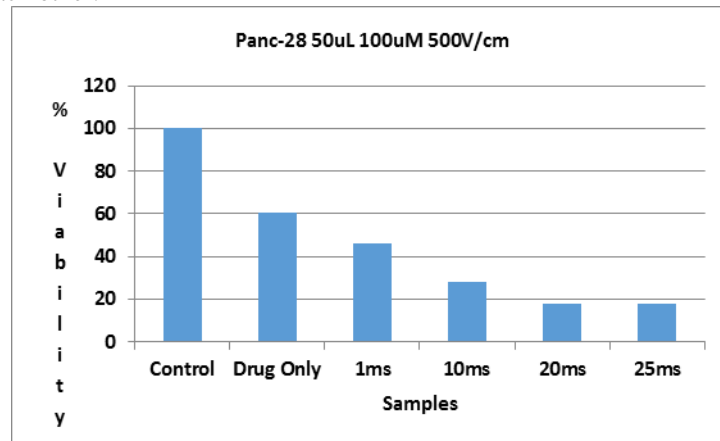


Fig. 6. Percent Viability of Panc-28 cells at Various Pulse Conditions for $50\mu\text{L}$ drug at $100\mu\text{M}$ concentration. Control indicates the sample with no treatment (neither the drug nor the pulses), drug only has only the drug (no pulses). The pulse intensity was $500\text{V}/\text{cm}$ in all the other samples.

Fig. 7 shows a comparison of the viability at 50 and 25 μ L volumes for the same concentration of 100 μ M. In this case, the drug saturation effect could be seen. The viabilities of drug only samples are 60.61% and 64.7% for 50 and 25 μ L respectively. For a reduction of 50% in the drug dosage, the viability only increased 6.75%. However, when electro-porated, for the same reduction in 50% of the drug dosage, the viability has increased by 32%. This indicates the advantage of using electrical pulses, for enhancing drug uptake.

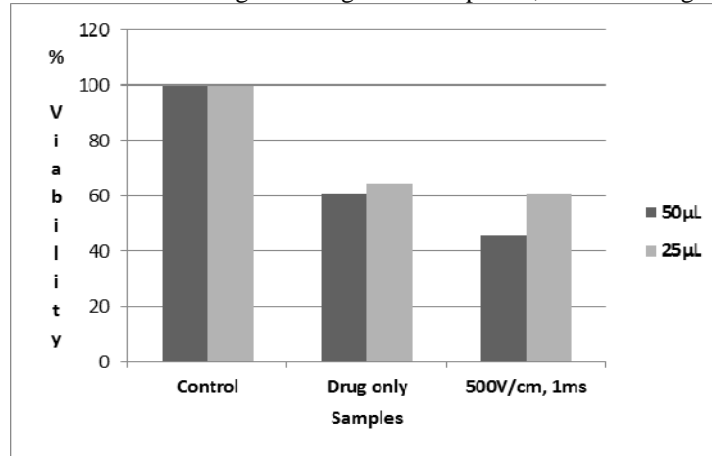


Fig. 7. Comparison of Viabilities at 50 and 25 μ L doses for the same 100 μ M concentration of the Gemcitabine.

Fig. 8 illustrates the viability at another set of pulse conditions. In this case, the samples were treated with eight 1200V/cm, 100 μ s pulses and 500V/cm, 25ms, 2 pulses. 50 μ L gemcitabine at 100 μ M concentration was used in both samples. The viability for the 1200V/cm sample is 18.32% and for the 500V/cm, 25ms, 2 pulses sample is 13%. This further illustrates that we can obtain high efficacy with low dose using electrical pulses than with drug only.

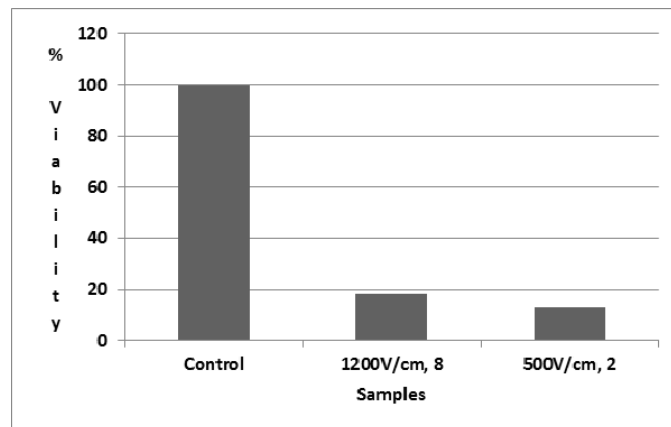


Fig. 8. Percent Viability of Panc-28 cells at Various Pulse Conditions for 50 μ L drug at 100 μ M concentration. Control indicates the sample with no treatment (neither the drug nor the pulses). The other two samples were treated with eight 1200V/cm, 100 μ s pulses and two 500V/cm, 25ms pulses.

DISCUSSION AND SUMMARY

Pancreatic cancer is one of most deadly cancers of humans. About three-quarters of pancreatic cancer arises in the head and neck of the pancreas (the anatomic parts through which the pancreatic duct runs just before it meets the duodenum). Some of these carcinomas arise in the body of the pancreatic organ, and less than ten percent arise in the tail of the pancreas (the tapering smaller “left” area, closest to the spleen). Fig. 7 shows the pitiful survival rate of pancreatic cancer—the incidence and the death rate are very close [13].

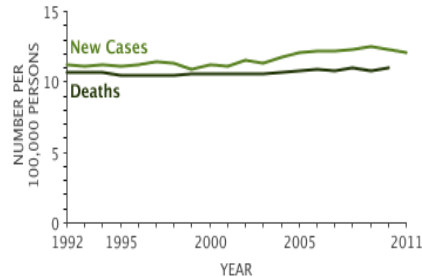


Fig. 9. Pancreatic incidence and death rate [13].

Each year more than 40,000 people in the United States are diagnosed with adenocarcinoma of the pancreas and more than twice that in Europe. Most of these people will have passed away by the end of the first year. The incidence of pancreatic cancer increases with age; most people are between the ages of 60 to 80 when they receive the diagnosis. Men have tended to be over-represented, though in recent years the gap between men and women has shrunk, possibly due to increased cigarette smoking among women. In the U.S., pancreatic cancer is 9th or 10th most commonly diagnosed cancer (depending on gender), but the fourth leading cause of cancer death in men and women. The median survival period from the time of diagnosis until demise is arguably the worst of any of the cancers. The median survival for untreated advanced cancer of the pancreas is about 3 1/2 months; with good treatment this increases to about six months, though many will live much longer. It is predicted that by 2030, it will double in number and 2.4x in death rates. This indicates that conventional surgery, chemo and radio therapies used for this cancer are inadequate and there is an urgent and critical need for alternate therapies.

One of the most important unsolved problems in pancreatic cancer therapy is increased tumor resistance to chemo treatment, using gemcitabine [14]. This increased resistance is a direct effect of defects in apoptosis. An alternative form of cell death, namely ECT is becoming and has the potential to be the first line of treatment for – due to its high efficacy and low toxicity.

ECT is becoming and has the potential to be the first line of treatment for – due to its high efficacy and low toxicity.

- With a viability of 60% with drug only, Panc-28 human adenocarcinoma pancreatic cells are resistant to the most commonly administered pancreatic cancer drug, gemcitabine at the used dose of 100 μ M.

- The various pulse conditions are successful in killing up to 85% of the cells. This indicates that the pancreatic cells are receptive to the electrical pulses at appropriate magnitudes and frequencies.
- Desired cell death could be obtained by optimizing the pulse parameters and the drug dosage.
- Electroporation enables enhanced drug uptake by opening pores in the otherwise impermeable membrane, due to the applications of electrical pulses.
- This could be used as a new mode of treatment for inoperable, and chemo-resistance pancreatic cancers.

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