

040304Background

Pancreatic cancer is one of the rarest cancers on the planet and yet is the fifth highest cancer killer due to its survival rate of an abysmal 3-5%¹. Although rare, this cancer is invariably fatal.

This year it is estimated that world-wide 213 000 people will be diagnosed with this disease and that 213 000 people will die because it². It is the only cancer that has a diagnosis rate less than the mortality rate.

Of the pancreatic cancers, adenocarcinoma of the pancreas is the most common. One reason that pancreatic cancer is so deadly is that it presents very little symptoms, and most of them can be attributed to various other, and more common, ailments. Some of its symptoms include:

- Malaise
- Nausea
- Diarrhea
- Weight Loss

All of these symptoms are very common in other, more common diseases and thus pancreatic cancer has been dubbed not only the “Problem of the 21st Century” but “The Silent Killer”³. By the time most tumours are diagnosed, they are not surgically resectable and a tumour of less than 1cm can often be fatal.

Diagnosis can occur through various mechanisms, usually stemming from unusual blood test results. The pancreas is responsible for a variety of metabolic functions including regulation of insulin and amylase, an enzyme responsible for the breakdown of carbohydrates. A blood test is able to show increased amylase levels which would signal abnormal pancreatic functions possibly indicating tumoural interference. After this, a laparotomy or a laparoscopy could be performed to determine if one is suffering from pancreatic cancer. If during the laparotomy a

biopsy shows the presence of cancer, the Whipple surgery will be considered. However, only 9%³ of patients qualify for this extremely invasive surgery. During the Whipple, the head of the pancreas, part of the gall bladder, part of the intestines and a portion of the stomach are removed; however, patients with surgically resectable tumours still only have a 24% chance of 5 year survival³. If surgery is not an option, Gemcitabin, a DNA chain terminating chemotherapy drug, will most likely be administered. While Gemcitabin is not usually successful at curing the neoplasm, it is fairly successful in helping palliate many of the symptoms of pancreatic cancer⁴.

Although alleviating some of the painful side effects of this cancer, these treatments are unable to cure cancer patients. It is evident a new treatment is needed.

Purpose:

To conceive of a new treatment that will be able to induce cell apoptosis and cell loss in human pancreatic cancer cell lines in vitro with no negative side effects on human beings in vivo. To create a treatment that will work not only as a palliative treatment but will be anti-cancerous as well as anti-tumoural.

Hypothesis:

A combination of a COX-2 (cyclooxygenase-2) inhibitor with the polyunsaturated fatty acid DHA will be beneficial in fighting adenocarcinoma of the pancreas. A recent study showed that DHA was able to induce cell apoptosis and cell loss in 75% of cells in the human pancreatic cancer cell lines Mia-Pa-Ca-2 after 50h⁵. A similar study was done on the colon cancer cell line HCA-7, where it was found that Celebrex (a commercial COX-2 inhibitor) and DHA were able to induce cell apoptosis at concentrations of less than 100uM and 150uM⁶. Another study has shown that DHA can sensitize various tumour cells to reactive oxygen species (ROS)-inducing anticancer agents⁷ and various studies suggest this to be true. The COX-2 inhibitor acts as an anti-inflammatory device to shrink the size of the cells and tumour. However, these commercial

COX-2 inhibitors have been found to cause heart disease and stroke in humans. Curcumin, naturally found in the spice tumeric, has no negative side effects on humans and acts as a natural COX-2 inhibitor. From this information, the hypothesis that curcumin and DHA will be able to induce cell apoptosis and cell death was drawn. It is also hypothesized that the DHA and curcumin will be synergistic and not just additive in their effects.

Materials:

- Cell lines Panc-1, Capan-2, BxPC3, HpCFII
- Curcumin
- DHA
- DMEM
- FBS
- Formalin
- Hematoxylin

Apparatus:

- Multiwell Culture dish
- Pipettes
- Beaker
- Incubator

Procedure:

This project was largely research as the majority of cancer projects are. It is crucial to have a fundamental understanding of the disease as well as current options before one can begin to hypothesize any sort of treatment or begin any experiments. However, a prototypic drug sensitive assay experiment was performed with the help of advisor Dr. Herman Yeger and Laura Meimari.

Experimental Procedure:

- 1) The cells were placed in a multiwell culture dish.
- 2) For 24 hours the cells were grown in a medium of DMEM and 15% fetal bovine serum.
- 3) After 24 hours, eight wells had a change in medium to act as a control.
- 4) After 24 hours, eight wells were treated with a combination of curcumin and DHA at 10uM
- 5) After 24 hours, eight wells were treated with a combination of curcumin and DHA at 2.5uM
- 6) After three days, cells were fixed with formalin.
- 7) Cells were stained with hematoxylin to act as a visual interpreter.

Observations:

BxPC3 and Panc-1 are strong growers due to the darker nature of the stain, which reflects their undifferentiated state. BxPC3 has some sensitivity to the combinations and this can be seen in the darker stains in the 10uM wells. It was observed that the cells became darker over time, and reached the darkest stage after 48 hours.

From a research perspective, it was observed that polyunsaturated fatty acids are usually able to induce cell apoptosis and that there is a change in oxidized GSH (glutathione). It was also observed that many gastrointestinal cell lines respond positively to a COX-2 inhibiting agent.

Conclusions:

The fact that certain cell lines became darker than others suggests that pancreatic cancer is a heterogeneous cancer that can have different growth potentials as well as phenotypes. BxPC3 agreed with the hypothesis however in the future more detailed assays should be done. All of the cell lines should have cytotoxicity tests performed to attain more conclusive results.

Research has demonstrated that COX-2 inhibitors have a beneficial impact upon cancer cells which suggests that the natural COX-2 inhibitor, curcumin, will have the same impact but without the negative side effects. It has been concluded that in combination, DHA and curcumin are potent anti-cancer agents and should be tested in vivo to see their true potential against adenocarcinoma of the pancreas. This project's focus was to find a treatment that could be potentially used as an anti-tumoural treatment for pancreatic cancer and according to research this combination could be one of the strongest anti-cancer agents currently available while having little or no negative side effects. Currently, more experiments are being performed testing various concentrations of DHA and curcumin both individually and in combination to test for increased cell apoptosis as well as a possible synergistic effect. In the future, this combination is expected to have many positive applications toward the treatment of pancreatic cancer with the added benefits of limited side effects and cost effective treatment programs.

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