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Determining Efficacy of Anti-cancer Drug from Detection in Plasma using the HPLC Method

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Abstract

Objective: To determine if the pattern of detection of an anti- cancer drug in plasma influences the progression of tumor size in mice and the extent to which the efficacy of the drug is mimicked in clinical trials.

Methods: Science research databases, such as MEDLINE (Proquest), were searched to find articles involving, “HPLC” and “anticancer drugs” with “plasma”. A lot of these articles that were found were published in the year 2000 or after. Eleven articles described the detection of their specific drug in the plasma of mice and showing the progression of the tumor size as well.

Main findings: When testing the DMXAA anticancer drug, the tumor growth was delayed when the dose started to move out of the plasma, meaning the retention period was over. This article wrote a follow up, indicating it moved into the clinical trials. Other articles depicted similar findings of their high drug levels retaining in plasma being proportionate to large tumor size.

Conclusion: The correlation found between the detection of the drug in plasma shows that plasma is rightfully used to determine the efficacy of an anticancer drug. With xenografts or patient similar stimulations, most drugs will replicate pre-clinical trial results in most patients. Using animal models to detect plasma drug levels in pre-clinical trials is valuable because plasma has the ability to reveal the future efficacy of antineoplastic drugs on tumors in clinical trials.

Keywords: Antineoplastic drugs, plasma, tumor, HPLC, anticancer, mice, pre- clinical trials.

Introduction:

Cancer is one of the biggest unknown medical conditions that scientists all over the world are still trying to cure because of the amount of people that die from it all over the world. The best treatments are the ones that work effectively and efficiently. Some treatments work, but there is

no definite cure just yet. There are many drugs that are in the process of being tested and clinically trialed for accurate results. A widely used detection method is analyzing the drug's retention period and percent in the plasma. If extraction and detection of the plasma creates a peak using the HPLC method, it is concluded that there is anticancer drug in the plasma, which will reduce the tumor.

This is an important topic because the testing the plasma of the subject being documented will portray how effective the drug is on decreasing the tumor. Chemotherapy dose is hard to find because there are many factors that play into each patient's dosage. Giving too much will induce drug toxicity and prevent all tumors from reacting in accordance with given protocol (Canal et al., 1998). Pharmacological knowledge helps with the dosage because it can help utilize anti-cancer drugs at their optimal level. There are several mechanisms for analyzing toxicity levels. Plasma drug concentration is the most potent pharmakinetic parameter because it accounts for physiologic and genetic characteristics (Canal et al., 1998).

Not only do plasma drug concentrations support toxicity findings, but also the efficacy of antineoplastic drugs on tumors. The drug concentration is the driving force of how effective the drug will be because the molecules of the drug bind to the proteins and lipids of the plasma, which will eventually lead them to their target cells (Zhang et al., 2019).

There have been many studies performed to determine the level of their tested drug in vitro and vivo organism models' plasma. Passing this step will enable researchers to patent the drug because it supports the end goal: to extend into pharmaceutical companies with an anticancer drug that is more effective than the previous sold ones. The most efficient way to proceed with plasma testing is the HPLC (high performance liquid chromatography) method. This method is a more advanced technique than all other chromatography, but at the basic level, it has the same

function: separate and distinguish compounds in a mixture. In detailed form, HPLC is a column chromatography that pumps solvent, which is the mobile phase, and detects the particles in it, which is the stationary phase. The results present a graph showing absorbance vs. time, which should create one peak where the drug is detected, allowing the retention amount of drug in the plasma to be known. HPLC allows performance with optimum resolution in minimum time. This resolution is influenced by parameters such as retention, selectivity and efficiency (Monmouth Junction 2016). This is the best method of detection because there are many different types of sample preparation, so depending on the solvent one may use, there's a variety of choices prior to the HPLC machine that can be changed to influence and change the results. Solid-phase extraction(SPE) and liquid chromatography with mass spectrometry (LC/MS) are some common methods that are used to prepare the sample being inserted into the machine(Monmouth Junction 2016). This is helpful because the HPLC machine can be modified to one's liking in order to obtain results supporting the thesis.

Methodology

In order to find the articles to start this paper, the database MEDLINE was used. MEDLINE (ProQuest) is a popular biology database that includes many journals of all things related to medical research and concepts in the science field. Due to the main topic of this paper being cancer and methods of research, it seemed to be a perfect fit to have all the information that was needed to be included in the report. At the beginning of the search for sources, only "HPLC" and "Cancer" was a basic key term used to find articles. Later on, "plasma of mice" became search terms as well because these in vivo models that scientists used were the basis of the paper. The whole paper was based on experiments researchers conducted with mice models and their plasma to keep the results controlled; different model organism might cause a source of

varying information. Also, articles that included other methods of testing the plasma were excluded because this paper was meant to only focus on the high performance liquid chromatography method. In all the articles that were chosen, an anticancer drug, the HPLC method of testing with the plasma and the results of the reaction of the mice from the drug was needed to be included in order for the article to be used. Most of these articles were searched for to be published after the year 2000, but if it was close to 2000 and seemed fitting in the paper, then the information from that article was used as well.

Literature Review:

` In a study with Zhao, Kestell, Ching and Baguley, 2002, an anticancer drug, DMXAA, was detected in plasma, which was compared to the biological activity of the tumor to analyze its effect in the tumor bearing mice. They limited confounding variables because humidity and temperature were constant for all test subjects, which were all female. Before oral administration was proceeded with, they were fasting for 16 h and they also were fasting 4 h after administration. The colon cancer 38 tumor was placed subcutaneously into anesthetized mice and were treated with drugs 8 days after implantation, measuring the tumor size three times in a week. There was one group that were left as the control group, so they were non-tumor bearing. The tumorous mice were either treated orally or through intraperitoneal (i.p) administration. At specific times, a blood sample would be taken from the ocular sinus and centrifuged and DMXAA plasma concentrations were measured using solid phase extraction and HPLC. The tumor was not cooperative with this drug when administered orally, which was seen when the tumor volume was measured and the plasma concentrations were tested. To assure the drug was working independently from other confounding variables, the non-tumor bearing mice were also

given the drug and had their blood sample concentrations determined as well through HPLC. The results of both samples are seen in Figure 1, which are put together to compare.

In another study by Rathinavelu, Alhazzani, Dhandayuthapani, et al., 2017, mice were collected and injected with tumors to understand the antiangiogenic activity of a vascular endothelial growth factor receptor- specific inhibitor drug. This drug was used so that blood vessels would cease formation and oxygen/nutrients could not reach the tumors to help them grow. The anti-cancer drug was used for many types of cancers. It was tested on female nude mice, which had human breast cells injected into their mid right flank that grew in two weeks. The experiment was controlled by temperature as well, so that many variables in the lab were not creating inaccurate results/conclusions (Rathinavelu et al., 2017). MUC-1 is a gene that is shown on apical surfaces of many organs, including breasts, so the levels of this in the plasma was used to assess ED1. The results and pictures show that the level of MUC-1 genes decreased in the plasma at the optimal dosage. The usage of xenografts in this experiment was a great merit because these models are the best replications for research being done in models to be considered in humans. Graphs and results were clearly formatted and explained in their captions to help the reader completely understand the data collected.

The drug Ecteinscadin 743 by Rosing et al.,1998, and hydroxytyrosol in the plasma by Ruiz- Gutierrez, Juan, Cert, and Planas, 2000, are two studies done by different scientists researching the effects of anticancer drugs in plasma. They both use solid phase extraction to prepare their samples because they found this was the best method because the flow rate can be controlled when separation of the mixture occurs. Also, these studies validate their methods with statistical tools, such as checking for the precision and accuracy with ANOVA, linearity, sensitivity of limit of quantification and selectivity by analyzing the peaks with its retention time

and testing many drugs to check the accuracy of the peaks. The results were found to show that an accurate and efficient method was developed to study the specific drug concentration in plasma. It is important to have this information because it is the way to be able to test a drug concentration in blood in animals in order to be able to apply that information into humans and produce new medicine and techniques for all the cancer we have in the world.

Most articles that were included were from the year 2000 and beyond because this is the most relevant and up to date information that can lead to an accurate conclusion. They all used HPLC as their key method of detection and very were careful with their confounding, independent and dependent variables. These studies were detailed and were supported with information about the techniques and processes of how plasma can be used to test drugs in vivo and in vitro models that can eventually be passed onto the pharmaceutical stage and delivered to patients. The methods a lot of these researchers use are similar, which is a sign that there are more famous ones, which might be more efficient procedures.

Discussion:

Comparing many of these articles used in this review, it is seen that the High Performance Liquid Chromatography machine was used in many studies. This machine is an essential portion of the whole study because it is set to test for the detection of the drug in the plasma. However, prior to this, sample preparation is needed and has to be done with trial and error based on the outcomes. The most common is solid phase extraction because it can be used for gas chromatography and HPLC, both methods being very sensitive and efficient. Due to plasma and the drug contained in it being charged, it is more common to use HPLC, but gas chromatography is also a viable choice when dealing with certain drugs.

All articles used the HPLC method for detection of drug in the plasma of either an organism or an in vitro model, which led to a conclusion that testing the plasma is an accurate method of seeing whether the drug is in the plasma. This would support the hypothesis because the drug proves to be in the plasma and is tested to show it works in shrinking the tumor size. However, the study by Lopez-Lazaro stated that there is a gap between the pre-clinical trials and the real clinical trials with human patients. Even though their belief is supported with statistics on death of cancer patients who took the drug candidate, it is not possible to create the assumption that the drug is the causation for this. The underlying fact that cancer is not always treatable in all patients when trying these new drugs is true, but the article blamed poor models, which excludes all the research that is being done with exceptional models. Researchers that build models closely resembling the human cancer are able to assume the high drug efficacy in vitro and in vivo models will mirror those in actual patients (Li 2019). Patient derived xenograft models are (PDX) are used in many advanced pre-clinical trials because they capture the outside and inside of the tumor heterogeneity. In the study by Rathinavelu, these grafts were used and the study supports this idea that the grafts create a more successful preclinical trial. As seen in Figure 2, the tumor volume and MUC -1 gene levels are directly proportional because in section b and d of the graphs, the groups vary in the same way. As the tumor volume decreases the MUC-1 gene level decreases, which was the original bioindication for the positive effect of the ED1 on the tumor(Rathinavelu et al., 2017). This further proves that the plasma levels are necessary to detect whether the drug is effective in targeting the tumor cells or not; this can be done directly or indirectly such as the case here.

There is one discrepancy in the two graphs because the control does not have the same MUC-1 level as the tumor, which shouldn’t be the case because the tumor keeps growing. This

could be an error that was decided not to be discussed in the article because it can be fixed with a few more trials, but for the majority of the graph, this pattern still applies. The study of DMXAA by Zhao was concluded to support the thesis as well. While they didn't use xenografts to model the patients, their data shown in Figure 1 exemplifies that the plasma was rightfully used to detect whether or not the drug decreased the size of the tumor. At the beginning of the article, it is noted that phase 1 of clinical trials have already been successfully completed, meaning humans have been interacted with this drug, so just as it worked in the mice, it also had a good outcome in humans. The purpose of the article was to see if orally or intraperitoneally administered drugs gave a better outcome, but in the process they also add evidence to support the thesis. Its half-life is longer when taken orally, so it doesn't react immediately and has a longer effect than needed, not producing the correct effect on the tumor. This is seen biologically and pharmacokinetically with the measurement of the tumor and the drug levels in the plasma. Figure 1(left) shows that the tumor size does not decrease by much even though the drug is administered. When compared with Figure 1(right), it is seen at that same drug level and type of administration, the DMXAA level stays in the plasma for a while and does not react quickly, ruining the chance of a great effect on the tumor. This proceeds to show that the plasma drug concentration is a great indicator for the actual effect on the tumor.

Limitations

Although there was a conclusive result, in most of the articles that were analyzed, there were not many limitations discussed, which is deceiving because there are limitations to every method conducted in science. An overarching limitation in many of the articles was that the HPLC machine was not described as sterile before using it. It might have been and not noted, but this is a common problem that many researchers come across during their work with this method

is that the peak is altered when reading the absorbance due to impurities from other previous usages of the machine. Also, in many articles, there was a discrepancy over which method of sample preparation should be used prior to detection using the HPLC method. In the study of the drug "Ecteinascidin 743" Rosing, solid phase extraction preparation was used, but caused difficulties when finding results. This was a limitation because the machine could not detect the concentration of the drug at the beginning of infusion at very low volumes of the drug, which occurs in other studies too. In these cases it could be useful to use liquid liquid extraction in order to prepare the sample to help the HPLC machine to be more sensitive when detecting drug amounts. In the study from Zhao, Ching, Kestell, and Baguley(2002) of the detection of DMXAA in tumor-bearing mice, the oral dose was less prolonged in the plasma compared to the intraperitoneal administration, which still worked because the tumor necrosis factor increased, but it might not be effective enough to diminish the patient's cancer, so the dose amount and schedule would have to be further studied.

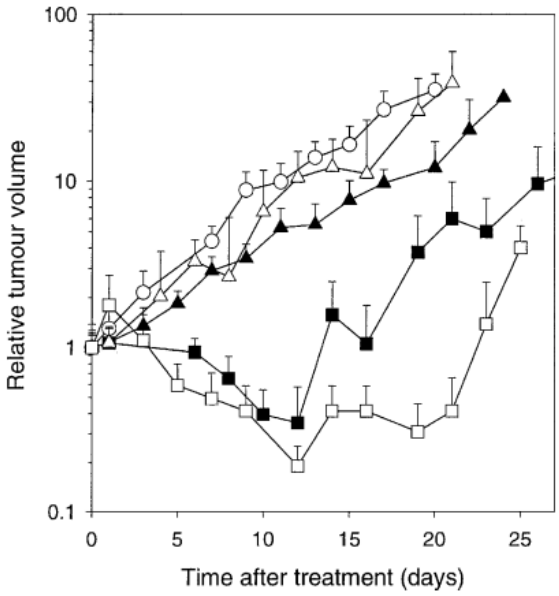
Summary:

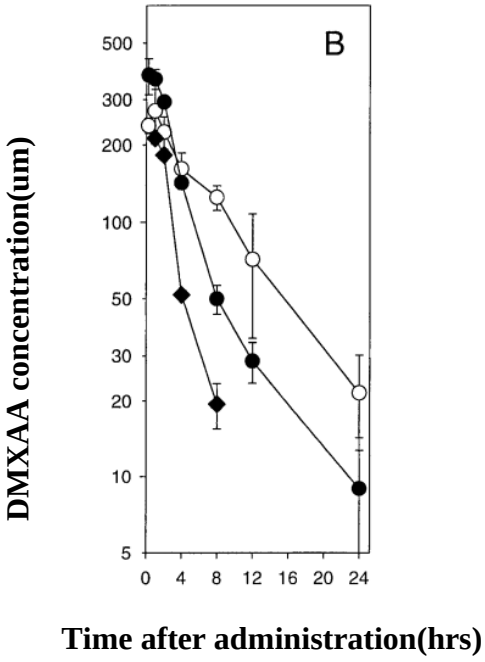
The purpose of this paper was to analyze the extraction of plasma and prove the detection of an anticancer drug in plasma using the HPLC method concludes that the drug decreases the size of the tumor. In summary of the findings, anticancer drugs are hard to make even with all the technology, but there are many out there. To say some don't work does not mean that none of them do because patients also influence the effect the drug will have on you. All patients have different types of cancer, which are located in a variety of tissues, so this causes a varying result in tumors when tested with a certain anticancer drug. Also, the demographics of each patient is different, so very few drugs will be sure to cure all cancer in every patient; each case is different and has its own needs. However, the hypothesis is supported to a high extent because the drug

retention period and retention amount in the plasma of models definitely can be shown with the use of HPLC method. The results will portray a graph, which can be compared with the tumor size in the model, analyzing the drug retention and tumor size. If there is a high amount of drug retention and the tumor size decreases, then the drug efficiency can be evaluated. Overall, with the correct sample preparation and usage of detailed models, the extraction and detection of an anticancer drug in plasma of models is assumed to resemble the efficacy of the drug in patients.

One thing to further study would be to continue analyzing drug retention in plasma with the use of gas chromatography(GC). This method has its perks over the HPLC method because volatile substances can be vaporized and detected with high sensitivity in this method. It would give the research a new method to check results or maybe even get more accurate results based on the type of drug being studied. Another possible study can be the detection in tissues where the cancer is present. Using the HPLC method, it can be determined with further studies if the tumor is decreasing if the actual tissue is analyzed. The plasma is detected for its retention period and amount, but the tissue will clearly portray whether or not the drug is targeting the right area of the tissue to shrink the tumor.

Appendix:





Zhao, Kestell, Ching, & Baguley. (n.d.). photograph, *Cancer Chemotherapy and Pharmacology*. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11855749

Figure 1(left): Tumor growth with no treatment (open circles), oral drug administration treatment(open triangles), intraperotineal treatment(closed squares), higher intraperotineal treatment.

Figure 1 (right):DMXAA concentration over time with closed circles(i.p) and open circles (orally).

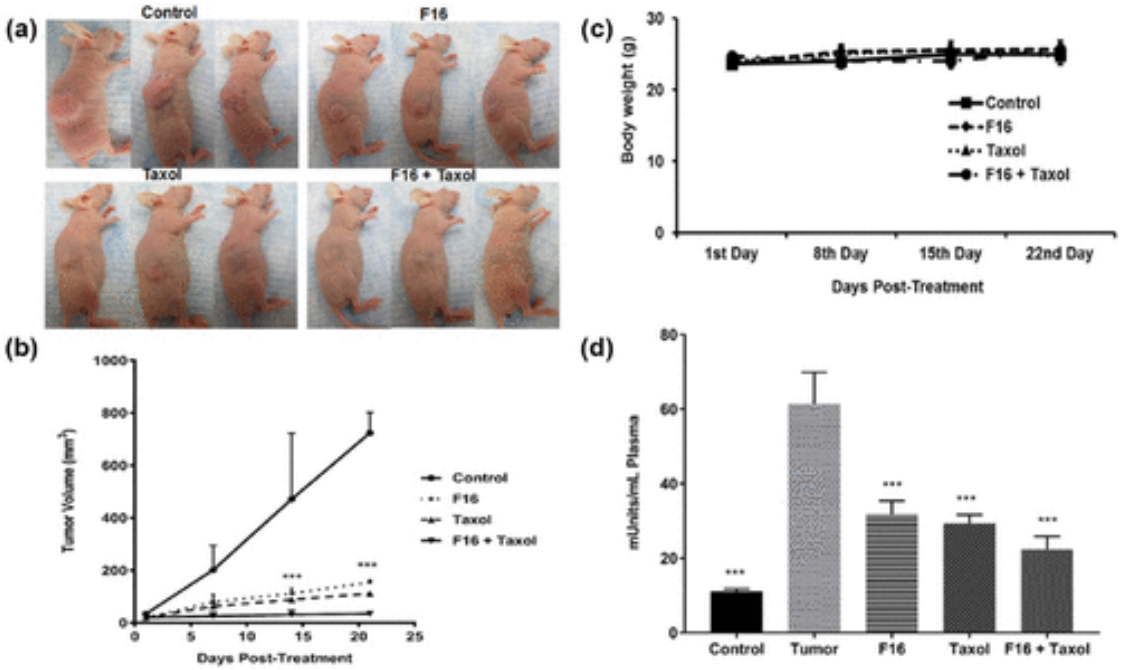


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Figure 2: b shows the tumor volume across all four groups and d represents the MUC-1 gene level in the plasma for the four different groups tested in the preclinical trials of the ED1.

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