ABSTRACT

An Evaluation of the Anticancer Effects of Triptolide in Pancreatic Cancer

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Pancreatic cancer is among the most lethal human malignancies with a 5-year survival rate of <5%. This adenocarcinoma is resistant to current chemotherapies, highlighting the need for more effective treatments. Recently, triptolide, a diterpenoid that has shown promise in other cancers, has been examined as a potential treatment for pancreatic cancer. The majority of triptolide study has focused on its pro-apoptotic effects through up-regulation of apoptotic pathways and down-regulation of inhibitory pathways. These studies have shown that triptolide is effective both in vitro and in vivo against pancreatic cancer cells. Also, triptolide has been shown to increase the effectiveness of chemotherapies pancreatic adenocarcinoma is usually resistant to when used in conjunction with them. The major reason that triptolide has not had much clinical testing is due to its poor water solubility. Minnelide, a water-soluble prodrug of triptolide, was created to hopefully harness the capabilities of triptolide for clinical use. It is my belief that Minnelide and triptolide should be studied to determine which regions of the molecule impart which anticancer effects. In this way, it may make it easier to determine the reasons behind potential toxic side-effects of the prodrug in humans.

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AN EVALUATION OF THE ANTICANCER EFFECTS OF TRIPTOLIDE IN PANCREATIC CANCER

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By

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TABLE OF CONTENTS

CHAPTER ONE

Introduction

Pancreatic ductal adenocarcinoma (PDAC), the most common form of pancreatic cancer, is currently the fourth most frequent cause of cancer-related deaths and one of the most lethal human malignancies overall. The current 5-year survival frequency for patients with pancreatic cancer is < 5%, making the detection rate almost equal to the mortality rate. This rate has been the same for over three decades even with advances in medication (Jemal et al., 2009). The current median survival rate is approximately four months (Burris and Storniolo 1997).

Current treatments available for pancreatic cancer are often ineffective and limited in the percent of patients they can help. The only available possibility for a cure for pancreatic cancer, surgical resection, is only possible in 10 to 20% of all patients. This is because pancreatic cancer's early stages are often asymptomatic, leading to a diagnosis at a later stage where resection is often not an option. In 40 to 50% of patients, the local cancer has advanced to a point where surgery is not possible. In the other 40% of patients the disease has already metastasized by the time it is detected. In the cases where surgery is not possible, current treatments are centered on palliative care (Warshaw et al., 1990).

Because surgery is often impossible, the current study of pancreatic cancer treatments focuses mainly on chemotherapy. This has proven difficult as pancreatic cancer is highly resistant to the majority of developed chemotherapies. The most potent

available chemotherapy, gemcitabine, was approved by the FDA in 1996. Since then, no drug has matched up to its effects. Gemcitabine works as a nucleoside analog for deoxycytidine triphosphate. It also has an active nucleotide form that works to inhibit the action of ribonucleotide reductase through a diphosphate. This inhibition decreases the deoxynucleotide pools, leading to an increased amount of gemcitabine in relation to its analog deoxycytidine. As this ratio increases, the amount of gemcitabine incorporated into DNA increases. This incorporation has been linked to a loss in cell viability (Plunkett et al., 1996). After treatment with gemcitabine, the median survival time of patients increases from approximately four months to 5.65 months. This means that gemcitabine gives an increased median survival time of less than ten weeks (Burris and Storniolo et al., 1997).

There is a need for new, potent chemotherapies for pancreatic cancer, so many potential treatments are being tested to try and find something that can affect this horrible disease. One in particular, triptolide has shown promise as an anticancer drug for many types of cancer including pancreatic cancer (Cui et al., 2012). Triptolide is an extract from the plant *Tripterygium wilfordii*, also known as the thunder god vine. This herb grows in the mountain regions of southern China and has been used for centuries as a treatment for arthritis. More recently, *Tripterygium wilfordii* has been studied for its anti-inflammatory and anti-autoimmune effects, specifically in Crohn's disease, kidney transplantation, and arthritis. This herb is currently undergoing phase 2b clinical trials (Liu et al., 2011).

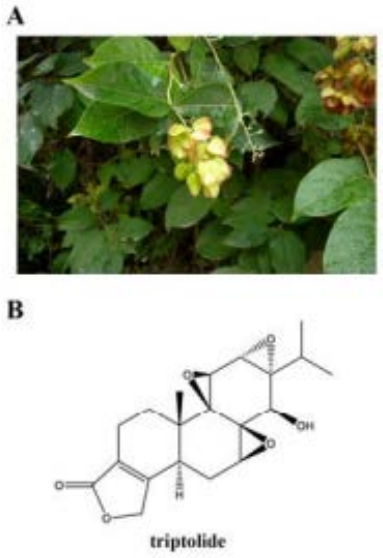


Figure 1: Picture A shows a *Tripterygium wilfordii* plant in bloom. Picture B is the diterpenoid triepoxide triptolide (Liu et al., 2011).

Triptolide is a diterpenoid triepoxide isolated from the roots of *Tripterygium wilfordii* in 1972 (Fig 1). More recently synthetic synthesis of this molecule has been performed. Triptolide shows broad spectrum anticancer effects including proliferation inhibition and apoptosis induction in vitro and metastasis inhibition in vivo. These anticancer effects occur through a myriad of inhibited and activated biochemical mechanisms. These mechanisms range from suppressing kinases to inhibiting molecular chaperones to transcription factor targeting and beyond (Liu et al., 2011). When triptolide was studied in over thirty pancreatic cancer cell lines, sensitivity to triptolide's effects was detected in all of them (Cui et al., 2012). The varied number of mechanisms triptolide works through in combination with its ability to affect virtually all pancreatic cancer cell lines make it a promising chemotherapeutic.

This literature review will explain the biochemical mechanisms through which triptolide has been shown to induce apoptosis, autophagy and inhibit angiogenesis in pancreatic cancer, how triptolide works in combination with other treatments for pancreatic cancer, and what recent advancements in triptolide could bring it to the forefront of chemotherapy for this deadly disease. It will also argue that the parts of the triptolide that cause such potent anticancer effects must be determined so that potential unforeseen effects of any triptolide prodrug in clinical trials can be attributed to a specific region of the triptolide molecule. Future clinical trials of triptolide prodrugs should also explore its synergistic effects when used in combination with other treatments.

CHAPTER TWO

The Mechanisms of Triptolide

As stated in the introduction, the anticancer effects of triptolide have been studied at the biochemical level enough to ascertain that many different pathways play a part in causing said effects. However, pancreatic cancer is extremely resistant to chemotherapy. This means that there is the potential for some of triptolide's many effects to not work in pancreatic cancer for any number of reasons. In order to ascertain the extent at which triptolide can impart its anticancer effects upon pancreatic cancer cells, it first must be studied thoroughly to see which pathways and mechanisms are affected by it. For this reason the majority of studies of triptolide in pancreatic cancer cells were focused on the biochemistry of how it worked. This research mainly focused on pro-apoptotic effects, but also worked to determine triptolide's pro-autophagic and anti-angiogenic effects.

Fas-mediated Pathway

The Fas-mediated apoptosis signaling pathway is so named because of the first step in the signal cascade—the binding of a transmembrane Fas Ligand, or FasL, to a transmembrane Fas Receptor, or FasR (Zacks et al., 2004). FasR and FasL have been noted to be expressed on the surface of pancreatic cancer cells. The binding of FasL to FasR causes signal transduction to recruit and bind Fas associated protein with death domain, or FADD, to FasL, forming the death-inducing signaling complex, or DISC (Fig 2). However, pancreatic cancer cells show a resistance to apoptosis. This seems to point to an inhibition of FasR-FasL binding. The mechanism by which this inhibition seems to

occur is the competitive binding of FasL to Decoy Receptor 3, or DcR3. This binding causes the amount of FasL available for binding to decrease, and therefore the ability for it to bind and signal apoptosis to decrease (Wang et al., 2012).

Triptolide's myriad of pro-apoptotic effects begins at the level of competitive DcR3 binding. Both in vitro and in vivo, Triptolide was shown to downregulate the expression of DcR3 in pancreatic cancer cells. This alone would allow more FasL to be available for binding to FasR, causing the strength of pro-apoptotic signaling to increase. In addition, triptolide has been shown to upregulate both FasL and FADD, allowing for more DISCs to be formed, and therefore a stronger apoptotic signal to be generated (Wang et al., 2012).

The formation of the DISC attracts pro-caspase-8 to the intracellular side of the complex so that it can be cleaved into its activated form, caspase-8 (Zacks et al., 2004). Caspase-8 has been shown to be upregulated in pancreatic cancer cells in response to triptolide treatment. This activation increases in both dose- and time-dependent manners (Wang et al., 2006). This is important because it shows that triptolide's effects on the Fas-mediated pathway cause a signal cascade that could account for its pro-apoptotic effects. Caspase-8 has multiple functions, but the one associated with the Fas-mediated pathway is its cleavage of pro-caspase-3 into the activated caspase-3 (Zacks et al., 2004).

Intrinsic Pathway

Unlike the straightforward way triptolide influences the Fas-mediated pathway, the intrinsic pathway of apoptosis is affected through a myriad of upregulations and downregulations. The majority of the actors in the inhibitory pathway are members of

the Bcl-2 family of proteins, which play important roles in the permeability of the mitochondrial membrane (Dudeja et al., 2009). One of these Bcl-2 proteins, the BH3 interacting-domain death agonist, or BID, is activated by the actions of triptolide on the Fas-mediated pathway (Wang et al., 2006).

BID is a death agonist of the Bcl-2 family of proteins, and its pro-apoptotic effects come from its BH3 region. The subgroup of BH3 death agonists BID is a part of is the BH3-only group. This region has been linked to pro-apoptotic effects in other Bcl-2 family proteins like Bik/Bbk and Blk. The BH3 region of BID differs from those of other Bcl-2 family proteins due to its location and BID's other important regions, BID seems to be distinct in the group of BH3 death agonists. The regions in question are two cleavage sites for caspase-8. When used in an intact state, BID has shown pro-apoptotic capabilities in very large doses in spleen tissue (Li et al., 1998). However, upon cleavage by caspase-8, BID becomes its activated form tBID, or truncated BID, and its pro-apoptotic effects increase greatly. Triptolide has been shown to increase the amount of BID cleaved into tBID over time (Wang et al., 2006). The mechanism which triptolide most likely increases BID activation is through the activation of caspase-8.

Mitochondrial Membrane Permeability

The mechanism by which tBID promotes apoptosis is through the recruitment of Bax and/or Bak to the mitochondrial membrane surface. There, the Bax/Bak proteins will group together and form a channel through the mitochondrial membrane, known as the Mitochondrial apoptosis-induced channel, or MAC (Dejean et al., 2010). This complex allows the release of cytochrome c from within the mitochondria. Pancreatic cancer cells treated with triptolide showed an upregulated expression of bax mRNA

(Zhou et al., 2008) and an increased amount of cytochrome c in the cytosol (Wang et al., 2012). These two findings coupled with the activation of tBID via triptolide point towards triptolide having a role in cytochrome c release via MAC formation.

Triptolide's activation of tBid and subsequent formation of the MAC is not the only mechanism through which it alters mitochondrial membrane permeability to promote apoptosis. One of these is the downregulation of Mcl-1 via miR-204. Mcl-1 is a part of the Bcl-2 family of proteins, meaning that it has an effect on mitochondrial membrane permeability. In pancreatic adenocarcinoma, Mcl-1 is seen to be over-expressed (Miyamoto et al., 2009). Because pancreatic cancer cells are resistant to apoptosis, Mcl-1 over-expression would seem to decrease mitochondrial membrane permeability and therefore attenuate apoptosis. Upon studying how Mcl-1 was expressed in normal cells, it was discovered that the miRNA, miR-204, regulated gene expression in normal cells (Chen et al., 2013). miR-204 would bind to the 3' untranslated region (3'UTR) of the Mcl-1 encoding gene, hindering its expression. In pancreatic cancer cells that had undergone treatment with triptolide, Mcl-1 underwent a time- and dose-dependent decrease while miR-204 levels increased. By inhibiting the expression of Mcl-1, triptolide favors an increase in mitochondrial membrane permeability.

The least understood mechanism influenced by triptolide in pancreatic cancer cells is the inhibition of 5-lipoxygenase, or 5-LOX. In normal pancreatic cancer cells, 5-LOX is over-expressed, and promotes survival. One elucidated mechanism by which this occurs is the activation of Bcl-2 through leukotriene B4 (LTB4) signaling triggered by 5-LOX (Zhou et al., 2007). Bcl-2, the protein that gives the Bcl-2 family its name, works against Bcl-2 proteins like Bak and Bax to keep the mitochondrial membrane less

permeable. In fact, Bcl-2 has been shown to attenuate triptolide's pro-apoptotic effects when upregulated in pancreatic cancer cells (Wang et al., 2006). Upregulation of Bcl-2 can be caused by the upregulation of 5-LOX or an increase in LTB4 present. Studies have shown that triptolide acts to downregulate 5-LOX and LTB4 production in a dose- and time-dependent manner in pancreatic cancer cells (Zhou et al., 2007). What makes this mechanism not well understood is the fact that as triptolide is administered to cells in a dose- and time-dependent manner, the amount of cellular Bcl-2 is not affected (Zhou et al., 2008). This means that even though Bcl-2 can attenuate triptolide-induced apoptosis, and Bcl-2 can be upregulated by LOX-5 and LTB4, the downregulation of either of these upstream pathway components has no effect on Bcl-2. This indicates Bcl-2 is being regulated by multiple pathways in pancreatic cancer cells, and that some of these pathways are not affected by triptolide.

Triptolide has yet another pro-apoptotic effect through the downregulation of the nuclear factor kappa-light-chain-enhancer of activated B cells, or NF-κB, and its subsequent effects on Bcl-2 family proteins. NF-κB has many protumor and prometastasis effects related to the survival of the cell, but the most poignant one for an examination of triptolide is its relation to the Bcl-2 family proteins Bx-cl and Bfl-1. Like Bcl-2 and Mcl-1, Bx-cl and Bcl-2 are pro-survival proteins that act by decreasing the permeability of the mitochondrial membrane. This decreases the amount of cytochrome c released into the cytosol for use in apoptotic pathways (Wang et al., 2006). Through two different pathways, triptolide downregulates NF-κB and therefore both Bcl-2 proteins it transcribes.

One of the two mechanisms inhibited by triptolide that downregulates NF-κB is through the alteration of the poly (ADP-ribose) polymerase-1(PARP-1) protein. PARP-1 mainly works against chromosome region maintenance 1 (Crm1). The main way PARP-1 activates NF-κB is by promoting its nuclear accumulation. This lets NF-κB work as a transcription factor for many different proteins like Bx-cl and Bfl-1. Crm1 on the other hand works by promoting the nuclear export of NF-κB. By exporting NF-κB out of the nucleus it cannot work as a transcription factor, inhibiting many pro-survival proteins. When a normal cell is exposed to proinflammatory or proapoptotic signals, PARP-1-dependent PARylation of the p65 subunit of NF-κB occurs, decreasing its ability to interact with Crm1. Without the ability to interact with Crm1, NF-κB migrates to the nucleus and performs transcriptional activities (Luo and Kraus 2012). In pancreatic cancer cells, PARP-1 is found to be overexpressed, increasing the resistance, proliferation and metastasis of pancreatic cancer. Triptolide acts upon PARP-1 by cleaving it into an inactive form. This decreases the nuclear accumulation of NF-κB, downregulating Bx-cl and Bfl-1 (Wang et al., 2006).

The second mechanism inhibited by triptolide that downregulates NF-κB is through the inhibition of specificity protein 1, or Sp1, a transcription factor. Sp1 has important effects beyond the transcription of NF-κB which will be discussed later. Sp1 is a transcription factor of both the p50 and p65 subunits of NF-κB. In pancreatic cancer, Sp1 is seen to be overexpressed, resulting in an upregulation of NF-κB. When pancreatic cancer cells were treated with triptolide, there was a marked decrease in Sp1 activity. However, the levels of Sp1 mRNA were not affected. This points to either triptolide directly or an upstream event inhibiting Sp1. This upstream event was determined to be

the Hexamine biosynthesis pathway—a shunt pathway that regulates signaling and transcription factors. This pathway increases the activity of O-linked β-N-acetylglucosamine transferase (O-GlcNAc transferase) causing it to glycosylate targets at a faster rate. One target of O-GlcNAc transferase's glycosylation is the Ser-484 of Sp1; this modification activates Sp1 allowing it to bind to promoter sequences and act as a transcription factor. Although the exact section of the HBP that is affected by triptolide in pancreatic cancer cells is unknown, the result is the inhibition of O-GlcNAc transferase activity. This inhibition leads to Sp1 not being glycosylated and therefore not acting as a transcription factor of the p65 and p50 subunits of NF-κB (Banerjee et al., 2013).

The last and most complex mechanism by which triptolide increases mitochondrial membrane permeability is through the inhibition of pathways surrounding Heat Shock Protein 70 (HSP70). HSP70 is a chaperone protein responsible for helping larger proteins fold correctly while being translated. In pancreatic cancer cells treated with triptolide, HSP70 was found to be downregulated—both protein and mRNA levels were decreased (Phillips et al., 2007). In pancreatic cancer cells, the inhibition of HSP70 only seems to affect apoptosis; autophagy is not affected by a triptolide-induced downregulation of HSP70 (Wang et al., 2006).

The mechanism by which HSP70 is activated in pancreatic adenocarcinoma cells shares similarities with the activation of NF-κB. HSP70 is transcribed in part by the promoter Sp1 which is activated through the HBP. This shunt pathway increases O-GlcNAc glycosylation, leading to the ser-484 of Sp1 to be glycosylated, activating it. This pathway is overexpressed in pancreatic cancer cells, leading to HSP70 also being overexpressed. Because of triptolide's effects on the HBP, Sp1 is inactivated and

therefore HSP70 is downregulated. However, triptolide works through multiple promoters in the case of HSP70 (Banerjee et al., 2013). Heat Shock Factor Protein 1, HSF1, is a transcriptional factor of HSP70 that binds to Heat shock sequence elements (HSEs) throughout the genome. This promotes the transcription of HSPs from many different areas of the genome. Triptolide acts upon HSF1by decreasing both the protein an mRNA levels within the cell, which points to the downregulation of transcription. This decreases the amount of HSF1/HSE binding throughout the genome, downregulating HSP70 (Banerjee et al., 2013).

The inhibition of promoters is not the only way that HSP70 is downregulated; miRNA also plays a part in the inhibition of transcription. Like in the case of the Bcl-2 family protein Mcl-1, a miRNA, miR-142-3, was found to decrease transcription of the DNA it bound to. miR-142-3 binds to the 3' UTR of the HSP70 gene, regulating the levels of transcription. Triptolide exposure induced miR-142-3 in pancreatic ductal adenocarcinomas. This shows that triptolide has multiple effects on the miRNAome of the pancreatic cancer cells it is administered to, potentially opening up an avenue for future research (MacKenzie et al., 2013).

HSP70 is shown to inhibit the activities of a pro-apoptotic ion and a pro-apoptotic protein—Ca^{2+} and cathepsin b respectively. Calcium is sequestered by the mitochondria for multiple reasons including the control and modification of signals and the induce formation of the mitochondrial permeability transition, or MPT (Gunter et al., 2004). In normal pancreatic cancer cells, cytosolic Ca^{2+} is attenuated to insure the MPT remains unformed. The protein that attenuates this Ca^{2+} is HSP70. Since HSP70 is downregulated in cells treated with triptolide, there is less HSP70 available to attenuate

Ca^{2+}. This allows the levels of cytosolic Ca^{2+} to increase, favoring the formation of the MPT (Dudeja et al., 2009). Cathepsin b is a cysteine protease stored in lysosomes throughout the cytosol. One of cathepsin b's functions is to increase the permeability of the mitochondrial membrane through its proteolytic activity (Ben-Ari et al., 2005). In order for cathepsin b to travel to the mitochondria and disrupt the membrane, it must first be released from its lysosomes. HSP70 has been shown to decrease the permeability of lysosomes in a similar fashion to the interaction of Bcl-2 and the mitochondrial membrane. By downregulating HSP70, triptolide increases the permeability of the lysosome, allowing cathepsin b to act upon the mitochondrial membrane (Dudeja et al., 2009).

The permeability of the mitochondrial membrane is one of the most complex things influenced by triptolide in pancreatic cancer cells. Through the upregulation and downregulation of Bcl-2 family proteins, transcription factors, ions, proteases and miRNAs, the intrinsic pathway of apoptosis is allowed to continue via the release of cytochrome c from within the mitochondria.

Post-Permeability Pathway

Cytochrome c is a protein found within the inner membrane of the mitochondria, and is the major effector shown to be related to the triptolide-induced permeability of the mitochondrial membrane. The formation of the MAC via tBid activation was shown to induce the release of cytochrome c from mitochondria (Li et al., 1998). Pancreatic ductal adenocarcinoma cells treated with triptolide showed a marked increase in cytochrome c present in the cytosol (Wang et al., 2012). This was linked to the effects of the intrinsic

pathway of apoptosis by inhibiting the upstream caspase-8 which was shown to decrease levels of tBid and caspase-9 (Wang et al., 2006).

Caspase-9 is the protein directly affected by increased cytosolic levels of cytochrome c in the intrinsic pathway of apoptosis. Upon release from the inner mitochondrial membrane, cytochrome c recruits Apaf-1 and procaspase-9 together. dATP induces the oligomerization of cytochrome c and Apaf-1 while simultaneously binding to procaspase-9. Procaspase-9 stabilizes dATP binding to the complex, which results in the cleavage of procaspase-9 into its activated form, caspase-9 (Jiang and Wang, 2000). Caspase-9 has been shown to be activated through the triptolide-induced intrinsic pathway of apoptosis in pancreatic cancer cells by comparing its activation to that of upstream and downstream components of the pathway (Wang et al., 2012) (Wang et al., 2006).

Caspase-3

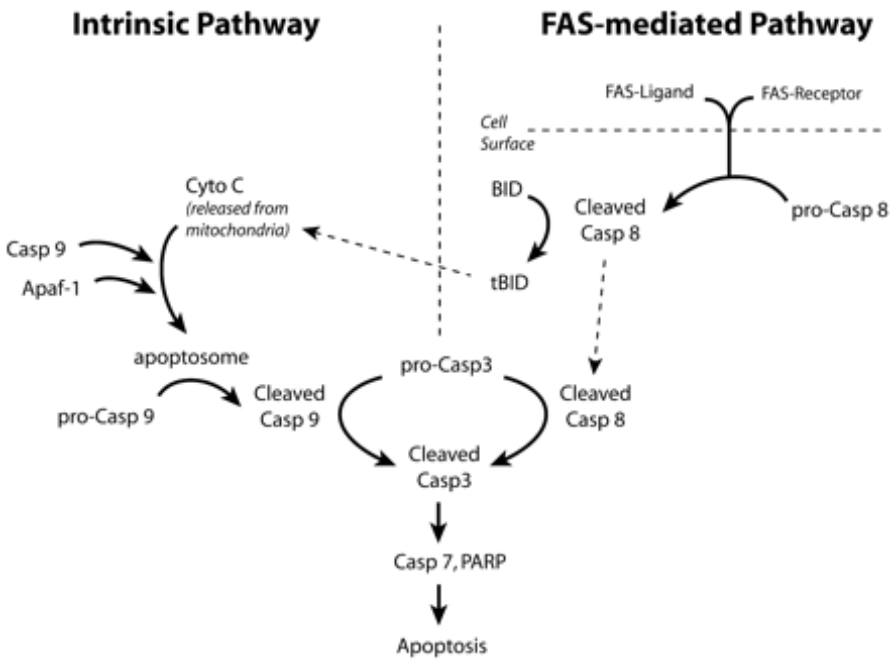


Figure 2: The simplified pathways of Fas-mediated and Intrinsic apoptosis. The mitochondrial portion is not shown (Zacks et al., 2004).

Caspase-3 is an effector (executioner) caspase, meaning that its function is to cleave proteins to trigger apoptotis within the cell (Wang et al., 2006). The expression of caspase-3 mRNA is upregulated in pancreatic cancer cells in response to triptolide treatment (Zhou et al., 2008). This effector caspase proves very important in the triptolide-mediated activation of apoptosis in pancreatic cancer due to its dual-activation by triptolide. The Fas-mediated pathway of apoptosis activates caspase-3 through the cleavage of its inactive form pro-caspase-3 (Wang et al., 2006). The intrinsic pathway of apoptosis also activates caspase-3 through cleavage of pro-caspase-3, but does so utilizing caspase-9 (Wang et al., 2006). Through activation of caspase-3, both of these signal cascade pathways work in conjunction to create the strongest possible signal for apoptosis in the cell.

Autophagy Signaling Pathways

The majority of research done on the biochemical pathways that triptolide influences to work against pancreatic cancer has focused on triptolide's myriad of effects on apoptosis. Recently however scientists have begun looking into other mechanisms of cell death that triptolide induces. Autophagy is a caspase-independent mechanism that occurs normally in cells to maintain them. This occurs for a few reasons in healthy cells: environmental stress, nutrient starving and malfunctioning cellular components to name a few. Through the use of lysosomes, autophagy is able to alter the intracellular environment to maintain homeostasis. However, signs of autophagy have been observed in larger numbers than normal within dying cells, potentially linking it to cell death (Mujumdar et al., 2010). Maintained and studied pancreatic cancer cell lines have many different properties and resistances when looking at the effects of triptolide on autophagy in pancreatic cancer, cell lines that show a preference for autophagy over apoptosis. Since autophagy is caspase independent, a knockdown of the dual-activated caspase-3 would greatly hinder triptolide's apoptotic capabilities while leaving the study of any potential pro-autophagic effects intact. In cell lines that favor apoptosis like MiaPaCa-2, Capan-1 and BxPC-3, this knockdown rescued cell viability. However, the cell lines S2-013 and S2-VP10, which favor the induction of autophagy, still showed a marked decline in cell viability after triptolide treatment. When these cells underwent a knockdown of autophagy-specific genes atg5 and beclin 1 as well as the caspase-3 knockdown, cell viability was maintained even after triptolide treatment. This shows that triptolide has an impact on autophagic pathways of cell death (Mujumdar and Saluja, 2010).

To determine which pathways triptolide affects to decrease cell viability through autophagy, two major pathways of nutrient starvation-induced autophagy were observed after treatment with triptolide: the Akt/mTOR/p70s6K and raf-1/Mek-1/ERK1/2 pathways. These pathways are common mechanisms by which autophagy is induced in cancer. The Akt/mTOR/p70s6K pathway is a negative regulator of autophagy. Akt is an upstream positive regulator of mTOR. Increased cytosolic ATP levels cause the phosphorylation and subsequent activation of Akt, when then starts a signal cascade (Mujumdar et al., 2010). Phosphorylated Akt will phosphorylate mTOR, activating it. The activated mTOR will then phosphorylate the p70 ribosomal protein S6 kinase (p70s6K), inhibiting autophagy (Aoki et al., 2007). Treatment of autophagy-induction favoring cells lines S2-013 and S2-VP10 with triptolide showed a downregulation of phosphorylated Akt and phosphorylated mTOR within a three-hour period. This shows that triptolide not only affects Akt either directly or through an upstream mechanism, but that this downregulation continues through the pathway, inhibiting a major negative regulator of autophagy (Mujumdar et al., 2010).

The ERK1/2 pathway is a positive regulator of autophagy in cancer cells. When a decrease in amino acids is detected by the pathway, raf and MEK1 phosphorylate ERK1/2, which phosphorylates Gα-interacting protein. This accelerates the rate at which the cell hydrolyzes GTP, which has been linked to increased autophagy (Aoki et al., 2007). In a similar manner to the Akt/mTOR/p70s6K pathway, triptolide treatment of S2-013 and S2-VP10 pathways upregulated the phosphorylation of ERK1/2 (Mujumdar et al., 2010). Through the inhibition of negative regulatory pathways of and activation of

positive regulatory pathways of triptolide induces autophagy in pancreatic cancer cells that have a penchant for autophagy-induced cell death.

Some of the mechanisms triptolide promotes cell death through have been reported to affect both apoptosis and autophagy. The autophagy pathway of Akt/mTOR/p70s6K promotes the inhibition of autophagy, but also works to inhibit apoptosis as well. Similarly, the activation of ERK1/2 has shown pro-apoptotic effects when kept activated for a long period of time (Aoki et al., 2007). The Bcl-2 family protein Mcl-1, which acts to promote survival via decreased mitochondrial membrane permeability, also acts to decrease autophagy-induced cell death in pancreatic cancer cells (Chen 2013). The pro-apoptotic ion Ca^{2+} has also been linked to increased activation of autophagy in pancreatic cancer cells (Mujumdar et al., 2010). This seems to point at triptolide activating autophagy through increasing mitochondrial membrane permeability as well as its effects on nutrition starvation-induced autophagic pathways.

Angiogenic Pathways

Triptolide has been shown to have multiple effects that promote autophagy through nutrition starvation-induced autophagic pathways. However, it has also been recently reported that triptolide plays a role in inducing nutrition starvation in pancreatic cancer cells through angiogenesis. Angiogenesis is the process by which cells create new capillaries to provide oxygen and nutrients to the surrounding tissue. Since cancer cells grow and multiply very fast, both of these are required. In order to make sure there is a large enough capillary bed to feed the tumor as it grows, angiogenesis promoting pathways need to be overexpressed. In pancreatic cancer, vascular endothelial growth factor, or VEGF, has seen to be overexpressed. VEGF works at least partially through

the tube formation of human umbilical vein endothelial cells, or HUVECs. Also, cyclooxygenase-2, (COX-2) which works to promote angiogenesis by inducing VEGF, is seen to be overexpressed in pancreatic cancer cells lines (Ma et al., 2013).

In the pancreatic cancer cell line PANC-1, triptolide has been shown to inhibit angiogenesis through COX-2 and VEGF. In cells treated with triptolide, the expression of COX-2 and VEGF was significantly decreased. This was shown by a decrease in mRNA levels of both proteins. The decreased amounts of COX-2 and VEGF caused the tube formation of HUVECs in vitro to be markedly decreased as well, meaning that there would be less capillary structure in the tumor (Ma et al., 2013). By decreasing the angiogenesis of the tumor, triptolide works to decrease the number of capillaries able to transport nutrients to the constantly proliferating pancreatic cancer cells. This nutrient starvation would then activate the nutrient starvation-induced autophagy. The sensitivity of this autophagic pathway is also greatly increased by triptolide's effects on regulatory pathways meaning that the signal for autophagy would be much stronger. This would lead to an even greater loss in cell viability.

CHAPTER THREE

Triptolide in Combination

The myriad of biochemical pathways triptolide alters in order to generate apoptotic, autophagic and angiogenic signals within cells are often the pathways that give pancreatic cancer cells their extreme resistance to treatment. The overexpression of Bcl-2, HSP70, NF-κB and many other proteins have been tied in some way to attenuation of the death pathways that occur in normal cells. Many treatments for pancreatic cancer have failed because of the extreme resistance to death these cells exhibit. However, if said treatments could be used in combination with a chemotherapy that hinders these resistances then potent anticancer effects could occur.

One of the earliest treatments for cancer was the use of ionizing radiation. However, pancreatic cancer has a very poor response to this type of treatment. The main use of ionizing radiation in pancreatic cancer has been reduced to providing palliative pain relief. When the effects of ionizing radiation were tested against the effects of triptolide in AsPC-1 cancer cells in vitro, it was found that 4 Gy ionizing radiation decreased cell survivability by 10% while triptolide dosed at 0.25 nmol/L decreased cell survivability by 48%. However, when the same doses of ionizing radiation and triptolide were added to the cancer cells together, cell survivability decreased by 79%. When tested in vivo on AsPC-1 tumors at similar doses over a 38 day period, triptolide treated tumors had a size of 0.2 g and radiation treated tumors had a size of 0.3 g. In combination, the tumor size was found to be even smaller, 0.08 g. This was compared to

the untreated tumor size of 1.7g. From a biochemical perspective, triptolide and radiation treatment in combination increased apoptosis both in vivo and in vitro more than either treatment separately. The in vitro effects were tracked by observing steps of the pathways: caspase-9, cytochrome c and caspase-8—which were all found to be increased by a greater amount. In vivo, the dual-activated caspase-3 activity was examined to determine which treatment worked the most effectively. Once again, the triptolide-radiation combination treatment provided the greatest increase in activation. This data points to triptolide and ionizing radiation having synergistic antitumor effects on pancreatic cancer both in vitro and in vivo (Wang et al., 2007).

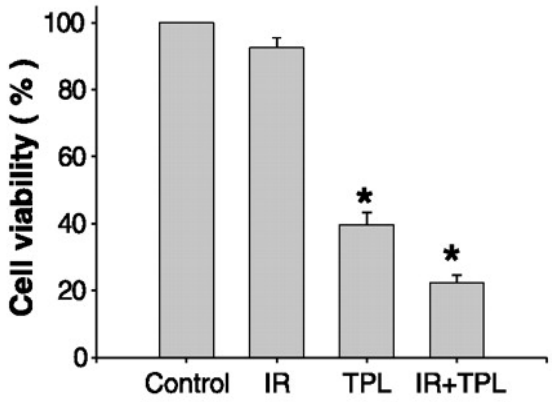


Figure 3: This graph shows the % cell viability of pancreatic cancer cells after treatment with saline, ionizing radiation alone, triptolide alone and ionizing radiation in combination with triptolide. The results show the greatest decrease in the combination treatment (* $p<0.05$) (Wang et al., 2007).

Once the use of ionizing radiation and triptolide in combination proved to be effective, researchers began testing triptolide for synergistic effects with other potent chemotherapies. One such chemotherapy was the lentivirus-produced 5-lipoxygenase short-hairpin RNA, or 5-LOX shRNA. A lentivirus is a retrovirus which can

permanently modify mammalian cells' genetic character. Lentiviruses produce RNA in extremely large quantities to ensure that the desired effect occurs on the effected cell. One type of RNA that can be manufactured by lentiviruses, shRNA, potently suppresses the gene it attaches itself to (Ravenscroft, 2008). One such shRNA, 5-LOX shRNA, has shown potent anticancer effects in xenograft mouse models. Biochemically, 5-LOX shRNA has a similar effect on the 5-LOX pathway in pancreatic cancer as triptolide. The effects of triptolide and 5-LOX shRNA on xenograft tumor weight were shown to be extremely similar as well. When 0.25 mg/kg triptolide and 5-LOX shRNA were used separately and in combination on xenograft tumors over a three week period, the results showed that the combination treatment was significantly better at decreasing tumor weight than either treatment was separately. This data points to triptolide and 5-LOX shRNA having synergistic antitumor effects on xenograft models (Ding et al., 2011).

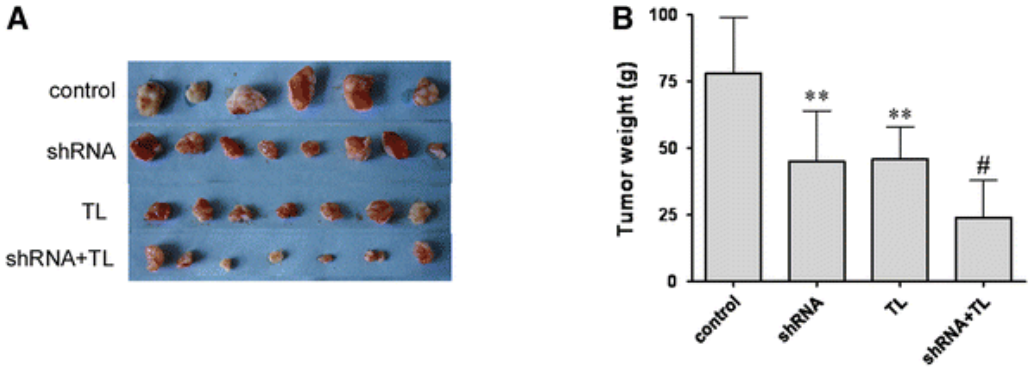


Figure 4: Picture A shows the decrease in xenograft tumor size of the different treatments visually. The combination shRNA and triptolide treatment shows the greatest reduction in size overall. Picture B shows a graph of different treatments against tumor weight. The combination treatment showed the smallest average weight (**$p<0.01$) (#$p<0.05$) (Ding et al., 2012).

The effects of triptolide on other drugs have also been studied with 10-Hydroxycamptothecin, an anticancer treatment with many issues. HCPT has shown promise due its broad spectrum of antitumor activity against many solid tumors. However, its water insolubility, structural instability, short half-life and high toxicity have made effective delivery of HCPT to tumors extremely difficult. In fact, at bodily pH HCPT converts from its lactone form to a carboxylate form that has increased toxicity and no antitumor capabilities. To keep effectiveness high and toxic effects at a minimum, HCPT has to be administered in small doses often. However, by placing HCPT into a micelle, a large amount of HCPT can be transported as if it was water soluble and be released slowly over time into target cells. These micelles target tumors through the use of folate-modified N-succinyl-N'-octyl chitosan (folate-SOC). The overall effect of these folate-SOC micelle HCPT is an overall decrease in tumor growth by half and a slightly decreased toxicity to noncancerous tissues (Zhu et al., 2013).

In vitro, HCPT effectiveness was tested against triptolide on PANC-1 pancreatic cancer cells. By looking at the activation of caspase-9, caspase-3 and NF-κB, it was determined that triptolide and HCPT in combination are more effective at activating caspase-9 and caspase-3 and inhibiting NF-κB in vitro than when used alone (Yang et al., 2011). This pointed to a synergestic effect between the two treatments in vitro. Additionally, the toxic effects of HCPT could be minimized by using a decreased dose in combination with triptolide and still get potent anticancer effects.

Artesunate is a drug similar to triptolide in that it is extracted from a Chinese herb. The herb in question is *Artemisia annua*, which has been used as an anti-malarial drug. More recently Artesunate has begun testing for its anticancer properties. These

properties include the inhibition of angiogenesis and anti-proliferative effects. In vitro, testing of both triptolide and artesunate on PANC-1 and CFPAC-1 cell lines showed that triptolide's effects were approximately ten times as potent; 100ng of triptolide had similar effects on expression of HSP20, HSP 27, HSP 60, caspase-3 activity, caspase-9 activity and annexin V to 100μg of artesunate. When used in combination, the effects on expression increased fourfold when compared to the two treatments alone. Also, in a xenograft tumor model triptolide and artesunate in combination had greater effects in combination than apart. However, no numbers of different average tumor weights or treatment time frames were given (Liu et al., 2013). This data points to an extremely synergistic relationship between artesunate and triptolide. However, without trials showing the treatment timeframe or potential toxic effects at the dosage levels, it is difficult to know if the data could point to a possible combination use in humans.

The final potential chemotherapy that has been studied with triptolide in pancreatic cancer is tumor necrosis factor-related apoptosis-inducing ligand, or TRAIL. TRAIL works similarly to TNF-α and Fas ligand in the way that it triggers apoptosis. However, TRAIL has a greater binding specificity than either TNF-α or Fas ligand. This means that TRAIL does not bind to normal cells and only induces apoptosis in cancer cells. However, pancreatic cancer cells are extremely resistant to TRAIL, possibly through the decoy receptor 3 overexpression present in them. Since triptolide has effects on decoy receptor 3, it was studied in combination with TRAIL to see if they work in a synergistic manner (Borja-Cacho et al., 2009).

In MIA-PaCa2, PANC-1, S2-VP10 and S2-013 cancer cell lines treated in vitro exclusively with TRAIL, the effects took large doses to become potent. At a dose of 20

ng/ml, MIA-PaCa2 and PANC-1 cell lines showed a maximum viability loss of approximately 75% and 25% respectively. However, S2-VP10 and S2-013 cell lines treated with the same dose showed no noticeable loss in cell viability whatsoever. When these same cell lines were treated with 50nM triptolide as well as the 20 ng/ml TRAIL, the results increased. MiaPaCa-2 and PANC-1 cell lines showed virtually no cell viability while S2-VP10 and S2-013 cell lines had their viability drop to approximately 25% and 10% respectively. Triptolide and TRAIL used together at the concentrations of 1.25 ng/ml TRAIL and 50nM triptolide showed an increase in apoptosis in all four tested cell lines and an increase in caspase-3 and caspase-9 activity in all four cell lines (Borja-Cacho et al., 2009). The way that TRAIL interacts with the cell is through binding to Fas receptors. Because triptolide seems to have a synergistic effect in combination with TRAIL, it would seem that the decoy receptor 3 downregulation allows TRAIL to bind, causing more potent anticancer effects.

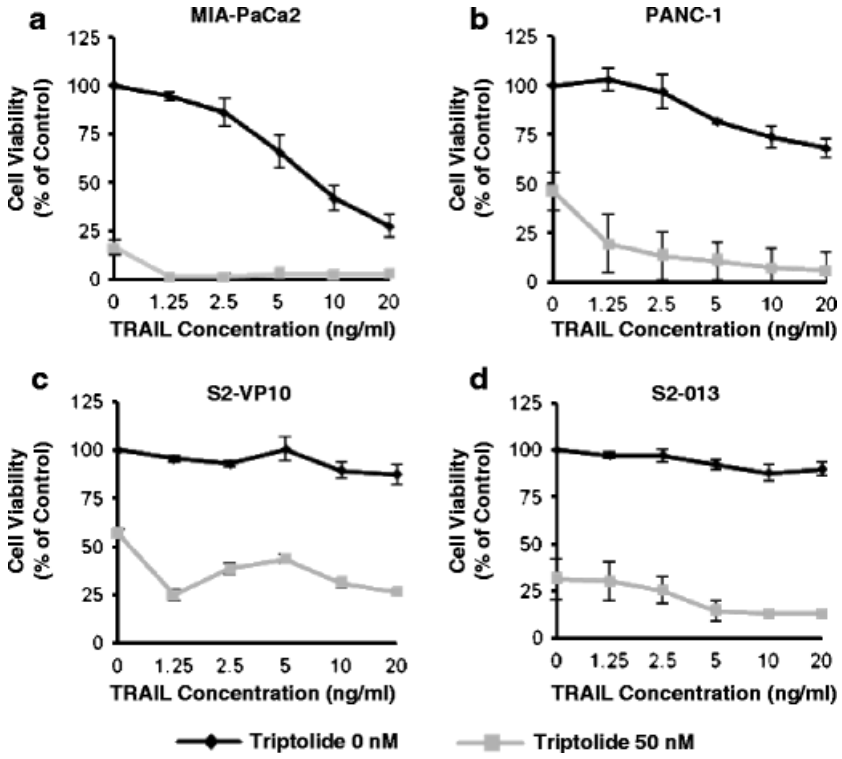


Figure 5: These four images show how cell viability decreases across four different pancreatic cancer cell lines when treated with TRAIL alone or in combination with triptolide. In every cell line, the addition of triptolide treatment greatly decreased cell viability (Borja-Cacho et al., 2009).

CHAPTER FOUR

Modified Triptolide: The Prodrug Minnelide

Triptolide has shown potent anticancer effects in a variety of pancreatic cancer cell lines through a myriad of mechanisms. It has also shown promise when used in conjunction with other types of treatment. However, unmodified triptolide has extremely limited clinical use because of one major issue: water insolubility. Triptolide normally is quite soluble in organic solvents. This makes it very proficient at passing through cellular phospholipid bilayers without the requirement of active transport (Chugh et al., 2012). However, clinically applicable drugs benefit greatly from water solubility. The site of major absorption in the human digestive tract is the gastrointestinal mucosa. The aqueous fluid that flows through this tract dissolves nutrients which can then be absorbed into the bloodstream through said mucosa. Water soluble drugs dissolve readily in this fluid, allowing them to be absorbed efficiently by the mucosa. This means those said drugs could be administered at lower doses than less-soluble counterparts and still get the same effect (Savjani et al., 2012).

To overcome this issue, a highly water-soluble analog of triptolide was synthesized. This synthesized molecule was named Minnelide (Chugh et al., 2012). The procedure by which triptolide was converted into Minnelide involves the removal of the 14-OH group and the replacement of it with a 14-O-phosphonooxymethyl group. This was performed using a four-step reaction. First, triptolide was treated with acetic acid, acetic anhydride and DMSO for five days at room temperature. This produced a

methylthiomethyl ether (-OCH_2SCH_3) group at the 14-OH site. This intermediate can be visualized as a white foam-like substance. When the intermediate was reacted with dibenzylphosphate and *N*-iodosuccinimide in dry methylene chloride, a dibenzyl ester derivative was created. This dibenzyl protective group was removed by reduction through the use of hydrogen gas on palladium and carbon to form the dihydrogen phosphate group. A simple reaction with sodium carbonate removed the hydrogens, causing the exposed oxygen groups to become negative. These negative oxygens stabilized with the sodium from the sodium carbonate, forming the 14-O-phosphonooxymethyltriptolide disodium salt known as Minnelide (Stella et al., 2001). The use of the 14-OH for conversion of triptolide to Minnelide not only increases water solubility—it also classifies Minnelide as a prodrug.

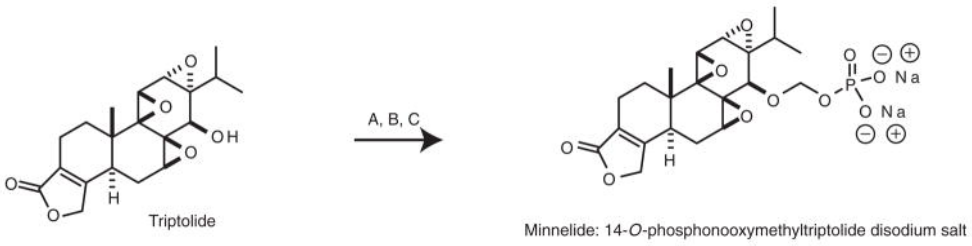


Figure 6: The process described to synthesize Minnelide out of triptolide. This removes the 14-OH and replaces it with a phosphonooxymethyl group (Chugh et al., 2012).

A prodrug is any medication that is administered in an inactive state and then later converted to its active form through metabolic processes. The reason that Minnelide is a prodrug is because a large portion of its anticancer effects have been linked to this 14-OH group. When the 14-OH group is replaced with a different functional group (an acetyl group was used in the study) the apoptotic effects of triptolide are abrogated. However, this functionality does not seem to be completely inhibited by the alteration of said 14-

OH group. At higher doses of 14-O-acetyltriptolide the pro-apoptotic effects occur, albeit in an extremely diminished fashion (Wang et al., 2006).

The process by which Minnelide can be converted back into a chemically active form involves the use of alkaline phosphatase. This molecule cleaves the phosphate ester group, which generates an O-hydroxymethyl intermediate. O-hydroxymethyl is an unstable intermediate and therefore will spontaneously react to release formaldehyde and triptolide. This conversion occurs as soon as alkaline phosphatase is present in solution in triptolide. The quick start to conversion is due to the fact that alkaline phosphatase is present in all tissues of the body, making post-absorption the first time Minnelide will be exposed to it. When testing how quickly Minnelide is converted into triptolide by alkaline phosphatase, it was found that the degradation half-life of Minnelide was two minutes. A rapid conversion of Minnelide into Triptolide like this allows for less of a delay between ingestion of Minnelide and its anticancer effects (Chugh et al., 2012).

To make sure that the synthesis of Minnelide from triptolide and subsequent degradation back to triptolide did not alter the produced triptolide and decrease its anticancer effects, Minnelide's in vivo biochemical effects were studied. It was found that degraded Minnelide still had effects on PARP-1 cleavage, Caspase-3 activation, NF-κB downregulation and Caspase-9 activation (Chugh et al., 2012). Also, genes that were related to the signaling of Bcl-2 and HSP70 were downregulated (Rousalova et al., 2013). Research showed that at a similar dose of 200 nM, both triptolide and Minnelide decreased pancreatic cancer cell viability in similar amounts in four different cell lines. This shows that Minnelide does not lose noticeable amounts of function from the synthesis and subsequent degradation back into triptolide (Chugh et al., 2012).

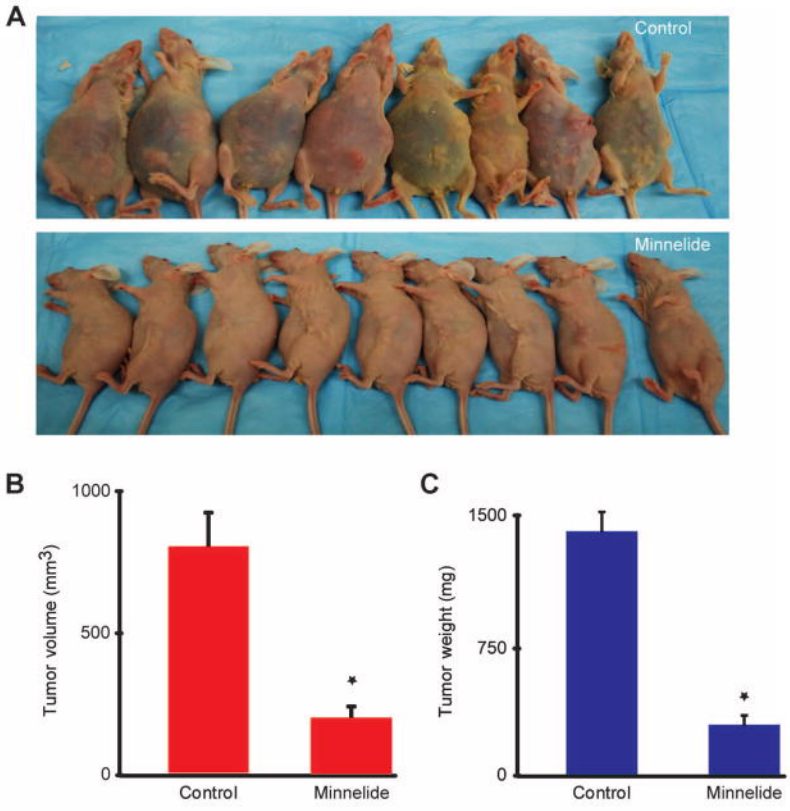
To hopefully predict the behavior of Minnelide in human trials, a sizeable amount of preclinical research has been done in vivo to see exactly what its effects are and what it targets in pancreatic cancer. One of the targets of Minnelide that was determined through this research was the $CD133^+$ population of tumor initiating cells (TIC). CD133 is a transmembrane pentaspan protein with no known function. However, it has been seen expressed on human hematopoietic stem cells, and is known to be a stem cell marker in both normal and cancer cells. In terminal ductal cells of the pancreas and in pancreatic ductal adenocarcinoma cells, C133 is expressed (Banerjee et al., 2014). These terminal ductal cells have enzymatic activity which has been associated with progenitor cells in many tissue types. The expression points to terminal ductal cells which express CD133 to be progenitors of pancreatic ductal adenocarcinomas that also express CD133 (Reichert and Rustgi, 2011). Because the $CD133^+$ pancreatic cancer cells will be earlier divisions of the progenitor cell, they make up the TIC. By isolating groups of $CD133^+$ and $CD133^-$ pancreatic cancer cells, it was shown that the $CD133^+$ group—which contains the progenitor cells and TIC—spread faster and are more resistant than the $CD133^-$ group. Upon treatment with Minnelide, both the highly resistant $CD133^+$ and $CD133^-$ populations within tumors were downregulated, showing a 60% decrease in tumor volume (Banerjee et al., 2014). The removal of TIC from the tumor will greatly hinder the tumor's ability to continue growing after the tumor becomes greatly reduced. Without strong progenitor cells, the TIC cannot create a tumor as quickly as it did before. Also, because Minnelide downregulates the TIC, it could also have anti-proliferative and anti-metastatic properties tied to anti-TIC effects.

In order to try and predict the level of effect Minnelide will have in people, many in vivo tests were run on mice to see its effects on tumor volume, weight, survival rate and metastatic properties. In initial testing to determine proper dosage of Minnelide, mice were injected with MIA-PaCa-2 cells and treated for 60 days with different doses of Minnelide or triptolide. After day 90 the mice were euthanized and data was collected on the tumors that grew. At the control value of 0.2 mg/kg QD, triptolide decreased tumor weight from 3291.3 ± 216.7 mg to 653.0 ± 410.9 mg and tumor volume from 2927.06 ± 502.1 mm^3 to 473.0 ± 291.9 mm^3. Of the eight mice treated at this dose, seven of them grew tumors. The most effective dose of Minnelide was 0.15 mg/kg BID. At this dosage, tumor weight was 373.0 ± 142.6 mg and tumor volume was 473.0 ± 291.9 mm^3. Of the ten mice treated at this dose, six of them developed tumors. One issue with this dosage is that the percent survival of mice dropped much faster than with QD doses. The QD doses took twice the time to even start showing similar drops in cell survival (Chugh et al., 2012). This data showed that Minnelide may have cytotoxic effects if the dosage was too high.

The determination of cytotoxicity in mouse models of pancreatic cancer is difficult due to the immunocompromised nature of the test subjects. In order to use xenograft models of cancer study in mice, the immune system of said mouse must be abrogated. However, this means that the cytotoxic effects of Minnelide cannot be studied in relation to the immune system. Instead, the level of liver damage is measured at certain doses. Because the liver filters the triptolide-laden blood, toxic levels of triptolide would be filtered into liver cells, making the effects magnified when compared to other parts of the body. As the liver is damaged, increased levels of bilirubin and alanine

aminotransferase (ALT) can be detected. At doses of 0.3 mg/kg QD for 29 days, Minnelide caused no significant differences in these levels. However, if the dose was increased to 0.6 mg/kg QD for 29 days, a significant increase in ALT levels in male mice was observed. This led researchers to use a dose in between, hoping to get the highest possible dose that still had minimal toxic effects. The dose that was tested for the rest of the in vivo studies was 0.42 mg/kg QD (Chugh et al., 2012).

At the dosage of 0.42 mg/kg QD, Minnelide was tested against saline injections to determine its effects on weight, volume, survival and metastasis. In a group of eight mice given S2-013 pancreatic cancer tumors with saline injections, the average weight of the tumor was 1387.5 ± 109.3 mg while the average volume was 799.6 ± 142.3 mm^3. All eight mice had ascites, spleen, diaphragm and abdominal wall metastases, and five of the eight mice had kidney and liver metastases. In a group of ten mice given S2-013 pancreatic cancer tumors with Minnelide treatment the average tumor weight was 290 ± 58.6 mg while the average volume was 199.8 ± 49.2 mm^3. Zero mice showed liver, kidney, diaphragm or ascites metastases. Two mice had abdominal wall metastases and one mouse showed spleen metastasis. The survival half-life of mice given AsPC-1 tumors was an average of 36 days. In mice that were given Minnelide treatment immediately upon tumor addition and continued indefinitely or stopped after 100 days of treatment the survival half-life did not exist, as no mice died after 385 days of study. When Minnelide treatment was administered only after the first mouse in the group died, no other mice died over a study period of 75 days. This data shows that Minnelide at a dose of 0.42 mg/kg QD has potent anticancer effects that decrease tumor weight and volume, and increase the survival time of mice treated (Chugh et al., 2012).



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Organ	Control	Minnelide
Liver	5/8	0/10
Kidney	5/8	0/10
Abdominal wall	8/8	2/10
Diaphragm	8/8	0/10
Spleen	8/8	1/10
Ascites	8/8	0/10

Figure 7: Picture A is a visual representation of the difference in tumor size and metastatic rate between the control group (upper row) and Minnelide treated group (lower row). Pictures A and B show that treatment with Minnelide significantly reduces tumor volume, weight and metastasis (* $p<0.05$) (Chugh et al., 2012).

The anticancer effects of Minnelide can only be understood when compared to the known anticancer effects of current chemotherapy treatments. Gemcitabine, currently the most potent pancreatic cancer drug, was tested against both a saline control and Minnelide on MIA PaCA-2 tumors in mice. The results showed that the values of control, gemcitabine, and Minnelide tumor volumes were 1437.5 ± 451.2 mm^3, 1371.4 ± 95.4 mm^3 and 587.5 ± 127 mm^3 respectively. Tumor weights of the control, gemcitabine, and Minnelide tumor were found to be 2150 ± 578.17 mg, 1371.4 ± 128.6 mg and 512.5 ± 120.2 mg respectively. This data shows that Minnelide is much more potent that the strongest available pancreatic cancer drug gemcitabine in a mouse model, and points to how potent it could possibly be in human trials (Chugh et al., 2012).

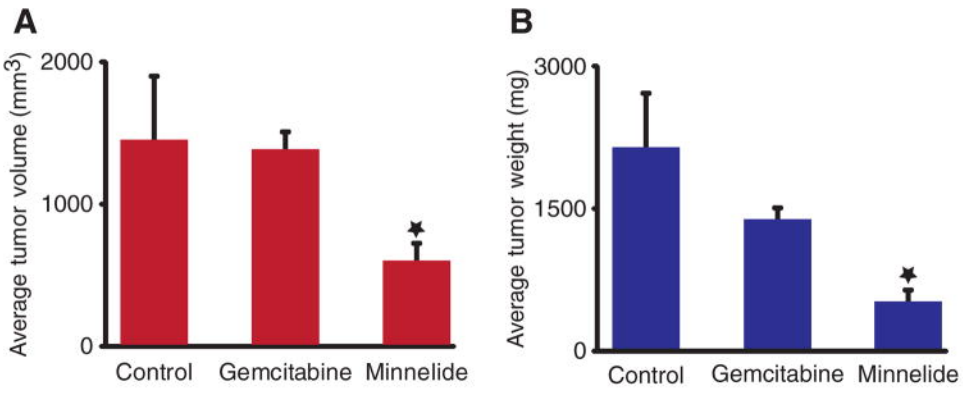

Figure 8: These results show the difference in antitumor effects between gemcitabine and Minnelide. Picture A shows the greater decrease in tumor volume Minnelide treatment causes in comparison to gemcitabine treatment. Picture B shows similar results, but for tumor weight instead (* $p<0.05$) (Chugh et al., 2012).

Because of the available documentation discussing the biochemistry, effects and minimal toxicity of Minnelide, it is currently undergoing phase I clinical trials. Because these trials are ongoing, the data is not available to the public. Human tumor-based xenograft models may point to the potential effectiveness of Minnelide in treating

humans. Tumors were taken directly out of patients and placed into immunocompromised mice. When Minnelide treatment was started as soon as the tumor was given to the mouse the tumor disappeared after less than 40 days, and did not grow back whatsoever over the remainder of the 90 day observation period. In mice that the tumor was allowed to grow to triple its size before Minnelide was administered, the complete disappearance of the tumor occurred after a similar amount of treatment time (Chugh et al., 2012). This data shows that Minnelide's efficacy on human pancreatic Cancer cells in vivo and in vitro. However, the mice used for xenograft models are immunocompromised, meaning that there are many interactions that cannot be studied by this model. Only the data from phase I trials will be able to show the true effects Minnelide has in humans.

Conclusion

From studying triptolide in many different types of cancer, its potent chemotherapeutic effects were discovered. These effects at the cellular level altered a myriad of pathways in order to cause increased apoptosis and autophagy while subsequently decreasing angiogenesis. It was found that not only did triptolide decrease the ability of cancer cells to proliferate and metastasize, but it also caused the tumors themselves to regress, sometimes completely. When studied in pancreatic cancer, one of the most lethal and resistant diseases known to man, it was found to still have these potent effects, shrinking and destroying tumors throughout every pancreatic cancer tumor and cell line it came in contact with.

This substantial ability for triptolide to perform even in extremely resistant cells has been studied, and it was found that triptolide decreases the resistance of pancreatic cancer cells to chemotherapy through many mechanisms. One major issue with current chemotherapies in pancreatic cancer is they cannot provide strong effects due to this resilience. Therefore, using triptolide in combination with other chemotherapies was studied, and in many cases these findings pointed to triptolide in combination being much stronger than either treatment alone.

However, triptolide on its own had a major hurdle to overcome in order to be used clinically; it lacked water solubility. In order to rectify this, a prodrug called Minnelide was formed that would impart this much needed water solubility to triptolide until it reached the bloodstream, where it would convert rapidly back into the potent anticancer molecule. Minnelide imparts this solubility by replacing the 14-OH of triptolide (Chugh et al., 2012). This OH of the molecule was found to give triptolide a large part of its anticancer effects, as removing it decreased these effects significantly. However, in high enough doses the effects of triptolide began to return (Wang et al., 2005). This points to multiple parts of triptolide playing a part in its anticancer effects. One issue with the current level of study is that the toxic side-effects of triptolide cannot be completely studied in mouse models. The immunocompromising used to make these models means that studies of triptolide in vivo cannot readily show the complex interactions it may have with and because of the immune system of the animal it is given to. Studies have shown triptolide to have minor immunosuppressant effects in humans, so there is interplay between these two systems.

At doses high enough to provide adequate anticancer effects, it is likely that toxic side-effects will occur. By mapping which anticancer effects are caused by different parts of triptolide, it should be possible to trace a toxic side-effect to a certain mechanism, and back to a part of the molecule. If the part of triptolide responsible for these problems is a region with only minor anticancer effects, then it could be possible to alter that region to stymie these effects without greatly decreasing triptolide's anticancer properties. For future research of both triptolide and Minnelide, I believe that research should be done on mapping anticancer effects of triptolide to different regions of the molecule, determining how these effects and regions relate to any potential side-effects, and if anything about triptolide can be altered to decrease the side-effects present. Also, I believe that future research of triptolide and Minnelide in particular should focus on its effects in combination with other available chemotherapies, as this has shown great promise in studies with multiple different treatments. A combination of treatments involving triptolide could be administered in smaller doses and still give strong anticancer effects, lessening any potential toxic side-effects. It is through the study of how to impart triptolide's anticancer effects to humans, while minimizing risk, that this plant extract could become a breakthrough chemotherapy for pancreatic cancer, and change the focus of non-surgical treatment away from palliative care and toward effective therapy aimed at tumor eradication.

APPENDICES

APPENDIX A

Abbreviations

FasL: Fas Ligand

FasR: Fas Receptor

FADD: Fas-Associated protein with Death Domain

DISC: Death-inducing signaling complex

DcR3: Decoy Receptor 3

BID: BH3 interacting-domain death agonist

tBID: truncated BH3 interacting-domain death agonist

MAC: Mitochondrial Apoptosis-Induced Channel

5-LOX: 5-lipoxygenase

LTB4: Leukotriene B4

NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells

PARP-1: poly (ADP-ribose) polymerase-1

Crm1: chromosome region maintenance 1

Sp1: specificity protein 1

O-GlcNAc: O-linked β-N-acetylglucosamine transferase

HBP: Hexamine biosynthesis pathway

HSP70: Heat Shock Protein 70

HSF1: Heat Shock Factor Protein 1

HSE: Heat shock sequence element

UTR: Untranslated Region

MPT: Mitochondrial permeability transition

APPENDIX B

Searches

PubMed Searches

Triptolide Pancreatic Cancer

Minnelide

Bid

Calcium and Mitochondria

Cathepsin b

Akt autophagy

PARP

MAC Bcl-2

Minnelide Cancer

Google Scholar Searches

TIC Triptolide

Water Solubility Prodrug

Gemcitabine pharmacology

Pancreatic Cancer surgery

Pancreatic ductal cells

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