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Ablational Therapies for Hepatic Tumors

By John M. Kane III, MD

The treatment of primary and secondary tumors of the liver represents a significant challenge to the global health care community. As a consequence of viral hepatitis, aflatoxins and alcohol, hepatocellular carcinoma is one of the most common malignancies throughout the world. Affecting approximately one million people per year, survival has been rather dismal, with a 25-90% three-year mortality based on the stage of the tumor.[1] In select patients, the only potentially curative therapies have been surgical resection or liver transplantation. In regard to secondary tumors, colorectal cancer remains the most frequent metastatic liver lesion in the United States. Liver resection is also potentially curative in certain patients, resulting in a 25-35% five-year survival. Unfortunately, many patients with primary or secondary tumors recur within the liver following resection and only a small proportion are candidates for reresection. Although usually only palliative in nature, many patients with metastatic neuroendocrine tumors also seek surgical treatment for symptomatic hepatic disease.

Rationale for Ablational Therapies

To understand the development of ablational therapies for hepatic tumors, one must appreciate the indications for and limitations of traditional surgical resection. Foremost, if hepatic resection of primary or metastatic tumors is undertaken for potentially curative intent, there must be no evidence of extrahepatic disease. Second, all hepatic disease must be "technically" resectable. Therefore, there should be no involvement of vital structures such as the hepatic artery, major bile ducts, or main portal vein. Third, there must be adequate functional hepatic reserve following resection. This is typically at least 20% of "normally" functioning liver parenchyma or more if there is hepatic dysfunction. Finally, given that liver resection is a major surgical undertaking, the patient should have minimal comorbid diseases in order to have an acceptable operative morbidity and mortality. Based on the above considerations, less than 25% of patients with primary or secondary liver tumors are candidates for surgical resection.

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In contrast to surgical resection, the fundamental advantage of ablational therapies is the ability to preserve uninvolved hepatic parenchyma. Given that treatment is directed specifically toward the tumor, only a small rim of surrounding liver is destroyed. As the majority of hepatocellular carcinomas arise from a background of cirrhosis, maximal conservation of parenchymal volume is essential for maintaining an acceptable treatment-related morbidity and mortality. This approach is also well suited to the characteristics of metastatic tumors of the liver; multiple lesions, bilobar disease, central location, or tumor in close proximity to major hepatic structures. Patients who have undergone prior liver resection with limited hepatic reserve can also be treated for recurrent disease within the remaining parenchyma. An additional benefit of ablational therapies is that they are more amenable to a minimally invasive approach.

In summary, ablational therapies allow for greater flexibility in patient selection. Some patients with poor tumor characteristics, limited hepatic reserve, or significant comorbid diseases that would preclude surgical resection may now be considered for ablational modalities. This approach is also well suited for the palliation of symptomatic neuroendocrine liver metastases. Therefore, ablational therapies potentially increase the number of patients who may be considered for treatment of primary or secondary liver tumors. At the present time, the most commonly used ablational techniques are percutaneous ethanol injection, cryotherapy, and radiofrequency ablation.

Percutaneous Ethanol Injection

Percutaneous ethanol injection is the oldest and most straightforward of the ablational modalities and has been used primarily for hepatocellular carcinoma. Under ultrasound guidance, a needle is placed into the tumor and approximately 1-10 mL of 95% ethanol is injected. The ethanol diffuses throughout the tumor, but extratumoral spread is usually limited by the surrounding cirrhosis. Treatments are repeated over several weeks to effect complete destruction of the tumor. An alternative approach for larger or multiple tumors is a single session with large volume ethanol injection. However, this technique often requires general anesthesia secondary to the intoxicating effects of the ethanol. The tumoricidal effects of ethanol injection are via two mechanisms; direct cellular dehydration leading to coagulation necrosis and vascular endothelial damage with thrombosis and subsequent ischemia.[2]

Benefits of Injection

The most striking benefits of percutaneous ethanol injection are the simplicity and minimally invasive nature of the procedure. Although it can be performed at the time of laparotomy, it is usually performed via a percutaneous non-surgical approach. Unfortunately, percutaneous ethanol injection is not without drawbacks. It is usually contraindicated in the setting of significant coagulopathy or ascites, which are fairly common to this patient population. Complications of percutaneous ethanol injection include intraperitoneal hemorrhage, hepatic parenchymal infarction, portal vein thrombosis, and tumor seeding of the needle tract. Unique to percutaneous ethanol injection are intoxication secondary to the ethanol and occasional biliary fibrosis after accidental intraductal injection. Despite these risks, the safety profile of percutaneous ethanol injection is excellent. In more than 1000 patients treated with percutaneous ethanol injection in the published literature, morbidity was less than 2.5% with no treatment related mortality.[2]

There is an evolving body of data in support of percutaneous ethanol injection as a potentially curative therapy for hepatocellular carcinoma that is comparable to surgical resection. Onodera and colleagues reported on a small series of patients with early-stage hepatocellular carcinoma who were treated with various modalities, including surgical resection and percutaneous ethanol injection. There was no significant difference in the

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three-year survival rates between percutaneous ethanol injection (91%) and resection (54%).[3] Kotoh and associates found no difference between surgical resection and percutaneous ethanol injection in regard to mortality (29% vs 39%) and recurrence (82% vs 70%) for solitary hepatocellular carcinomas less than 2 cm.[4] There are three key points in this study which deserve mention. First, patients selected for percutaneous ethanol injection were usually those with poor hepatic function or advanced age. Therefore, these patients were more debilitated than the resection candidates. Second, all intrahepatic recurrences in both groups were at sites remote from the original tumor. Finally, the procedural cost (excluding hospitalization) was 18 times greater in the surgical resection group.

In a large study of percutaneous ethanol injection for hepatocellular carcinoma by Livraghi and coworkers, five-year survival was 37-40% for single lesions less than 5 cm, 30% for lesions greater than 5 cm, and 26% for multiple tumors.[2] They also found that five-year survival decreased with worsening Child class (A, 47%; B, 29%; C, 0%). They noted that most of the Child A patients died of tumor progression, in contrast to complications of cirrhosis in the Child C patients. Therefore, percutaneous ethanol injection may have a limited effect on survival in patients with hepatocellular carcinoma and advanced hepatic disease. Finally, in a review of the available literature on surgical resection vs. percutaneous ethanol injection for single hepatocellular carcinomas less than 5 cm, the five-year survivals were comparable at 49% vs. 48%, respectively.[2]

Cryotherapy

Knowledge of the detrimental effects of freezing on both normal and malignant tissues dates back to ancient times. Early attempts at tumor cryotherapy were limited by the inability to obtain the extremely low temperatures necessary for complete tissue destruction. The advent of liquid nitrogen-cooled cryoprobes has resulted in more predictable and uniform tumor freezing. At the temperatures achieved with modern cryoprobes (-196°C), there is formation of both intracellular and extracellular ice crystals that produce direct cellular destruction and local tissue ischemia.

At the present time, cryotherapy is usually performed via an open technique. As with surgical resection, the liver is completely mobilized and vascular control is obtained. Using intraoperative ultrasound, the target lesions and their relationships to the major intrahepatic structures are visualized. Under real time ultrasound guidance, cryoprobe placement is performed using a modified Seldinger technique. A needle is placed into the lesion, followed by a guidewire, dilator, and finally the cryoprobe. To minimize complications, it is essential that the cryoprobe passes through a portion of uninvolved hepatic parenchyma prior to entering the tumor. For larger lesions, multiple probes can be placed simultaneously. The liver must also be insulated from the surrounding intra-abdominal structures to prevent inadvertent local injury. During freezing, the progression of the peritumoral "ice ball" is monitored by ultrasound. The goal is for the outer rim of the ice ball to exceed the tumor by at least 1 cm. For lesions adjacent to major vascular structures, there is occasionally incomplete freezing due to a "heat sink" effect from the rapid flow of warm blood. This effect can be minimized with temporary hepatic inflow occlusion at the time of freezing. The tumor is allowed to thaw and the freeze-thaw cycle is often repeated for a total of two to three times.

Given the large size of the cryoprobe, bleeding from the probe tract can occasionally be problematic. Cold-induced injury may occur to either the bile ducts or poorly insulated extrahepatic structures. Other complications include pleural effusion, cold-induced arrhythmias, and biloma. One of the most feared acute complications is a major fracture of the liver while the parenchyma is still frozen. Additional complications unique to cryotherapy include a transient postoperative thrombocytopenia, myoglobinuria occasionally associated with acute renal failure, and a syndrome of "cryoshock" consisting of multiorgan failure, coagulopathy, and disseminated intravascular coagulation.[5] A world survey by Seifert and Morris revealed an overall mortality of 1.5% for hepatic cryotherapy.[5] Although cryoshock was observed in only 1% of all patients, it accounted for 18% of all treatment-related deaths. It has been suggested that the development of cryoshock may be associated with multiple freeze-thaw cycles.

Recipients of Cryotherapy

The results of cryotherapy for primary and metastatic liver tumors are somewhat clouded by the fact that this modality is almost always reserved for patients with surgically unresectable disease. Consequently, this select group should have more advanced disease and a poorer prognosis as compared to surgical candidates. In addition, cryotherapy has often been used in combination with more traditional treatments such as resection, chemotherapy, or chemoembolization. In a study of the role of cryotherapy in primary liver malignancies, the one-, three-, and five-year survival rates for cryotherapy alone in 78 patients were 64%, 40%, and 27%, respectively.[6] For the treatment of hepatic colorectal metastases, Tandan and associates critically reviewed the available literature comparing cryotherapy to surgical resection.[7] The median follow-up for the cryotherapy studies was 12-29 months with overall and disease-free

survivals of 33-64% and 22-29%, respectively. In contrast, several large studies of surgical resection consistently had 20-40% five-year survivals. Given that the primary goal of cryotherapy for hepatic neuroendocrine metastases is palliation, results have been more promising. In a small study by Siefert and associates, 92% of patients with neuroendocrine metastases treated with cryotherapy were alive and mostly asymptomatic at a median follow-up of 13.5 months.[8] There was only one death which was not treatment or tumor related.

Radiofrequency Ablation

Radiofrequency ablation of hepatic tumors has developed in response to the limitations of cryotherapy. The large size of the cryprobes, the problem of cold injury to adjacent structures, and the risk of hemorrhage have necessitated an open approach to therapy. In addition, it has been proposed that heat may be a more predictable tumoricidal insult as compared to cold. The effects of tissue heating are well described: irreversible protein denaturation above 49°C, coagulation at 70°C, and desiccation at 100°C.[9] Radiofrequency ablation uses high frequency alternating current to generate tissue temperatures from 70°C to 90°C. Under ultrasound guidance, the tip of an electrode is placed into the tumor and current is applied for approximately 2-10 minutes. Given the small size of the electrodes, this technique can easily be performed percutaneously.

Maximal lesion size with monopolar radiofrequency ablation electrodes is limited to approximately 1.5 cm. Unfortunately, increasing the power applied to the electrode does not significantly increase lesion size as the flow of the current becomes limited by decreased conduction through desiccated tissues at the tip of the electrode. Therefore, several modifications of the technique have been developed to increase the maximal lesion size. These include inflow occlusion to minimize the "cold sink" effect of hepatic blood flow, multiple electrodes, cooling of the electrode tip, electrode tip irrigation with saline, and catheter tips with a deployable "umbrella" array of electrodes.[10]

Complications have been extremely rare with radiofrequency ablation. Given the small size of the probes and the coagulative nature of the therapy, the risk of hemorrhage is minimal. In a large study of 123 patients by Curley and coworkers, complications following radiofrequency ablation occurred in 2.4% of patients with no treatment-related mortality.[11] Although radiofrequency ablation is relatively new, early results have been promising for hepatocellular carcinoma, and to a lesser extent, for metastatic tumors. A recent study by Rossi and colleagues used radiofrequency ablation to treat 39 patients with hepatocellular carcinoma and 11 patients with metastatic liver tumors.[12] For the patients with hepatocellular carcinoma, median survival was 44 months, which corresponded to a 40% five-year survival. Of note, 41% of these patients developed recurrent tumor. However, only 13% of all recurrences were at the site of the previous radiofrequency ablation. For the patients with metastatic tumors, one patient remained disease free one year after treatment. In the study by Curley et al, post-treatment tumor recurrence was only 1.8% in 48 patients with hepatocellular carcinoma and 75 patients with metastatic tumors.[11]

Conclusions

At the present time, surgical resection remains the "gold standard" potentially curative therapy for primary and secondary liver tumors. However, the ablational modalities fill an important niche in the armamentarium of the physicians treating these patients. Due to poor location, underlying hepatic dysfunction, or comorbid diseases, only a minority of patients with hepatic tumors are considered candidates for surgical therapy. In addition, both primary and metastatic tumors have a high incidence of post-resection intrahepatic recurrences. Common to all of the ablational techniques is the ability to maximally preserve uninvolved liver parenchyma. They are also readily amenable to a minimally invasive approach. This allows for greater flexibility in patient selection for therapy. There is increasing evidence to suggest that percutaneous ethanol injection is comparable to surgical resection for the treatment of hepatocellular carcinoma. Although the role of cryotherapy and radiofrequency ablation in the treatment of hepatic tumors has not been completely defined, early results have been encouraging. Current interest in ablational techniques includes modification to laparoscopic approaches, ablation-assisted resection, and the use of microwave and laser catheters. With future technological innovations, the majority of patients with hepatic tumors may one day be successfully treated with non-resectional ablational techniques. *(Dr. Kane is a Fellow in Surgical Oncology at Roswell Park Cancer Institute, Buffalo, NY.)* ❖

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Dendritic Cells in the Treatment of Prostate Cancer

By Georgi Pirtskhalaishvili, MD, PhD, and Michael R. Shurin, MD, PhD

Cancer of the prostate is the most commonly diagnosed cancer in men and the second most common cause of cancer death. Despite significant advances in diagnosis, surgery, and radiation therapy in the past decade, a cure is possible only when the disease is localized within the prostate gland. The only effective treatment for advanced cancer of the prostate (PCa) is hormonal therapy, introduced more than 50 years ago, which does not provide complete cure. Chemotherapy is largely ineffective, since prostate carcinoma is a slow-growing tumor; only a small fraction of cells proliferate at any given time.[1] On the other hand, advanced PCa is a compelling target for immunotherapy because this approach does not require a high proliferative index. Prostate cells express more than 500 unique gene products which could serve as potential therapeutic targets. PCa thus offers potentially unique antigens, which may be particularly suited for the generation of specific antitumor immune responses following cell-based and/or cytokine-based immunotherapies.

Background

It is well known that cytotoxic T lypmhocytes ($CD8^+$ T cells, CTL) are the most powerful effector antitumor component of the immune system. Therefore, the aim of each antitumor vaccination is to induce a strong cytotoxic T cell response against the tumor antigens. However, T cells are unable to recognize unprocessed proteins. For activation, they require the presentation of antigens in conjunction with MHC molecules. The stimulation of naïve T cells also requires the presence of costimulatory molecules. Antigen-presenting cells such as B cells and macrophages cannot induce primary immune response without prior activation. However, dendritic cells (DC), the most potent antigen-presenting cells, are able to stimulate naïve T cells and mount primary immune responses.

DC, first described in 1973,[2] are significantly more potent than macrophages and B cells in their ability to stimulate T cells. Unlike monocytes, DC are able to induce the generation of cytotoxic T cells in the absence of $CD4^+$ helper cells.[3] A single DC can stimulate 100-3000 T cells and requires only a minimal amount of superantigen to generate a significant lymphocytic response.[4] Thus, a great interest in DC biology for tumor immunology during recent years is understandable. When the problem of DC generation in sufficient numbers was solved,[5,6] DC-based therapy was explored for cancer treatment. Pulsing of DC with synthetic tumor-associated peptides may induce an effective antitumor immune response.[7,8] DC can also be pulsed with a tumor lysate[9] or RNA[10] in order to induce specific immune reaction against tumors. All of these approaches have certain limitations when considered for use in humans, since the preparation of tumor lysates or extraction of tumor antigens requires a large amount of solid tumors. Another approach is a direct injection of DC into the tumor, where DC pick up tumor antigens in situ and present them to T cells. This approach confirmed its effectiveness in animal models including prostate cancer model (Nishioka et al. 1999, unpublished data).

Since PCa expresses a number of antigens, different peptides can be used for DC pulsing. Peshwa and associates employd prostatic acid phosphatase (PAP) peptides to pulse DC.[11] Activated cells were used to generate prostate-tumor specific $CD8^+$ cells, which were able to lyse prostate tumor cells in vitro. Prostate specific antigen (PSA) derived peptides were used by others for the generation of $CD8^+$ T cells which were able to lyse PSA-expressing cells.[12]

Clinical and Experimental Data

A clinical trial using DC therapy for PCa was undertaken at Northwest Hospital in Seattle, WA.[13] Autologous DCs were cultured from the peripheral blood mononuclear cells obtained from prostate cancer patients. The authors used two peptides for loading into DCs in this study. They were derived from prostate-specific membrane antigen (PSMA) and designated as PSM-P1 and PSM-P2. Five groups were formed from 51

patients with hormone-refractory prostate cancer. Two groups of patients received only peptides (PSM-P1 and PSM-P2), respectively, a third group was treated with DC only, and 4th and 5th groups received DC pulsed with peptides PSM-P1 and PSM-P2, respectively. Patients received four or five doses of the tested substance at 6-8 weeks intervals. Seven partial responders were identified based on National Prostate Cancer Project (NPCP) criteria. Two patients were from peptides-only groups (Groups 1 and 2), and five were from peptide-pulsed DC groups (Groups 4 and 5). There was no responder in the DC-only treated group. Side effects were minor. Immunologic monitoring studies showed an increase of T cell response to the appropriate PSMA peptides in patients in groups 4 and 5. No significant response was observed in groups 1, 2, or 3. A Phase II clinical trial started in January 1997 involving 107 patients.[14] Participants received a total of six infusions of autologous DC pulsed with PSM-P1 and PSM-P2 cocktail at six-week intervals. Overall, out of 95 evaluable patients, 11 (11.58%) had a complete response and 25 (26.32 %) had a partial response. Twelve of 19 patients (63%) with hormone-refractory metastatic PCa (stage D2) survived for more than 600 days. These results, though remarkable since effect was achieved in patients usually insensitive to the conventional modes of treatment, still demonstrate somewhat limited efficacy of the administered therapy.

One reason of the limited efficacy of immunotherapy in PCa patients is associated with the local or systemic suppression of the DC system by the PCa-derived factors, resulting in inhibition of immune responsiveness. Troy and colleagues examined the presence of DC in the prostate cancer and found significantly less DC in cancer tissue in comparison to normal prostate or benign prostatic hyperplasia (BPH).[15] Bigotti and coworkers found inverse correlation between the number of prostate cancer infiltrating DC and the histopathological grade of the PCa, with grade 5 tumors (which carry the worst prognosis) virtually devoid of antigen-presenting cells.[16] It is a well-known fact for many tumors that the presence of DC is correlated with a better prognosis.[17] The low number of DC within the prostate tissue may be due to low immunogenicity of PCa, (most prostate cancer cells lack MHC Class I antigens18), as well as the result of active suppression of DC by PCa-derived factors. Indeed, the coincubation of prostate cancer cells with DC resulted in death of DC19, which was documented in both human and animal models. The local or systemic suppression of the generation, function, and survival of immune cells induced by the PCa-derived factors and resulting in inhibition of immune responsiveness may limit the efficacy of immunotherapy in PCa patients.

Since prostate cancer caused active suppression of DC, the possible mechanisms of this protection were examined. So far, interleukin 12 (IL-12), interleukin 15 (IL-15), CD40 ligand (CD154), and TNF-α were studied for this purpose. IL-12 is a strong proinflammatory protein, stimulating INF-γ production by T and NK cells.[20-22] IL-15 induces T cell proliferation and promotes IL-12 production.[23] Together with IL-12, IL-15 has been shown to stimulate NK cells to produce INF-γ and TNF-α.[24,25] CD154 binds to CD40 on DC leading to the increased survival in cultures, probably secondary to increased expression of the anti-apoptotic proteins of Bcl-2 family.[26,27] TNF-α is involved in the regulation of cell death and proliferation, and induces the production of proinflammatory cytokines IL-1β and IL-6.[28,29] We found that the stimulation of DC with CD154, TNF-α, IL-12, or IL-15 resulted in the increased resistance of DC to the PCa-induced apoptosis.[30] Animal experiments are in progress to explore the effectiveness of modified DC therapy in murine prostate cancer.

Conclusion

DC have demonstrated their efficacy in the treatment of advanced prostate carcinoma, and their use will expand during the coming years. Until now, peptide pulsed DC were used mainly systematically. It is possible that intratumoral administration of DC will also be tested in the nearest future, which may produce a more specific response for a given patient, although experimental studies need to be carried out before the conducting of clinical trials. Since it was established that PCa causes active supression of DC, we expect that in coming clinical trials DCs will need to be modified before infusion to protect them from PCa-induced apoptosis. Alternatively, DCs may be administered together with cytokines, which may also enhance the antitumor activity of the host immune system. *(Dr. Pirtskhalaishvili is a resident in oncology and Dr. Shurin is on staff at Departments of Urology and Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA.)* ❖

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Tissue Inhibitors of Metalloproteinases

By Daren B. Filsinger

The role of matrix metalloproteinases (mmps) and tissue inhibitors of metalloproteinases (TIMPs) is extremely complex and poorly understood. TIMPs, cannot simply be viewed as inhibitors of the extracellular matrix (ECM) degrading enzymes, MMPs, but as regulators of growth factors, tumor invasion, metastasis, angiogenesis, and apoptosis.[1-3] The function of TIMPs in cancer is therefore aggressively researched and debated.

Matrix Metalloproteinases

MMPs belong to a family of zinc endopeptidases capable of degrading numerous components of the ECM. Most MMPs in cancer are probably not produced by the cancer cells themselves, but by local stromal and inflammatory cells. The cancer cells produce a stimulator for MMP release, known as tumor cell-derived collagenase stimulating factor (TCSP) or extracellular matrix metalloproteinase inducer (EMMPRIN).[4] MMPs are then secreted as proenzymes and activated by cleavage and subsequent exposure of the substrate binding pocket which contains Zn^{++}.

Tissue Inhibitors of Metalloproteinases

Currently four endogenous TIMPs are known (TIMP-1,-2,-3,-4). They are each approximately 25 kDa in size and have a highly homologous N-terminal domain. This domain is responsible for the irreversible inhibition of the active site of metalloproteinases. The C-terminal domains have less homology and confer different specificities and affinities for each MMP and other molecules. Blavier and associates have summarized the diverse functions of TIMPs as 1) either direct or indirect inhibition of vascular endothelial cell proliferation; 2) degradation of insulin-like growth factor-binding protein-3, releasing active IGF; 3) cell cycle and migration inhibition by intracellular signaling of an intact ECM, which is maintained by TIMPs; 4) indirect activation of MMP-2 by TIMP-2 via binding the metalloproteinase in close proximity to its membrane-bound activator enzyme; and 5) suppression of apoptosis by TIMP-1 in malignant Burkitt cells, potentially by a TIMP receptor.[3] A recent study demonstrates TIMP-2 and synthetic matrix

metalloproteinase inhibitors (MMPIs), but not TIMP-1, induce apoptosis in human T lymphocytes.[5] Depending on the cell line and specific inhibitor, TIMPs and MMPIs can have either inhibitory or stimulatory effects on cell cycle. This summary demonstrates the complexity of endogenous TIMPs and the controversy of their therapeutic usefulness.

Because multiple MMPs are notably higher in cancerous tissues, therapeutically exerting an imbalance in favor of TIMPs is, at first glance, of potential benefit. The stage and type of cancer may dictate how effective TIMPs may be. Aggressive cancers and extensive metastasis may overwhelm the effects of medicinal TIMP. Furthermore, little is known about the clearance of TIMPs but a short half-life in serum prevents systemic use and has lead to an interest in TIMP modification to increase half-life and gene therapy to induce overexpression of TIMPs in cancer cells.[3]

Synthetic Matrix Metalloproteinase Inhibitors

In order to overcome the obstacles of endogenous TIMPs, research has turned to synthetic inhibitors, the MMPIs. The goals in mind are to create the ideal inhibitor with oral bioavailabilty, an increased half-life, and specific activity only blocking the matrix degrading activity of MMPs while eliminating potential adverse effects like apoptotic inhibition and growth factor activation. Synthetic inhibitors work by entering the substrate binding pocket of MMPs and act as a zinc chelator. Early MMPIs, like batimastat, were broad-spectrum and effective against all matrix metalloproteinases. The use of batimastat in blocking tumor invasion in pre-malignant intestinal tumors resulted in a 48% decrease in tumorigenesis in mice.[6] The same study by Goss and colleagues demonstrated TIMP-1 as having far different effects, some of which were deleterious in mice. This again proves the complex nature of endogenous inhibitors. Newer synthetic inhibitors maintain a zinc chelating ability but are selective for specific MMPs. Whether this is of benefit has yet to be determined, because in most cancerous states mutiple MMPs are elevated and no specific matrix metalloproteinase has been linked to cancer. Groups like the gelatinases (MMP-2 and MMP-9), however, have been implicated.[7]

Recent Studies

Although most MMPIs have not reached human clinical trials, drugs like marimastat are leading the way in human trials determining efficacy, side effects, and dose, while other synthetic inhibitors are being modified and animal tested. Being noncytotoxic drugs, MMPIs have the potential to be used in combination therapy and elicit few side effects.

Some MMP inhibitors appear to eliminate one known side effect that others may elicit while maintaining anticancer effects. Preliminary results of a small study by Drummond and associates compared the effects of two different unnamed broad-spectrum inhibitors and two unnamed selective inhibitors on tumor growth (melanoma) and ability to cause tendinitis, a common side effect of some MMPIs.[8] Only one of the four tested maintained anticancer efficacy and did not cause tendinitis. The inhibitor with these results was one of the broad-spectrum inhibitors with activity unlike most broad-spectrum inhibitors. It has the ability to block the release of TNF-alpha. The other broad-spectrum inhibitor, like most, lacks the ability to bind to tumor necrosis factor-alpha convertace (TACE), which itself is a metalloproteinase. Inhibiting the release of the cytokine, TNF-alpha, decreases the possibilty of developing inflammatory side effects like tendinitis.[9] The results of this small study indicate that selective inhibitors may not be efficacious against tumor growth and anti-TACE activity is required to eliminate inflammatory side effects.

In a more extensive study, a potent, selective MMPI, AG3340, demonstrated that selective inhibitors are effective against tumor growth.[7] AG3340 was tested against human prostate, colon, lung, breast, and glial tumors that were grafted to mice. AG3340 is a synthetic inhibitor which is specific for the gelatinases and MMP-13 and -14 and has anti-TACE activity. Dose-dependent inhibitions of growth were observed in colon and lung models, while in breast cancer models the minimal dose demonstrated maximal inhibition. Tumor growth was also inhibited in glioma and prostate tumor models. The most significant results appeared in colon, lung, and glioma models with 65-79% growth inhibition compared to controls ($P < 0.05$). With early treatment, AG3340 also reduced angiogenesis in colon tumors. Potentiated efficacy was observed with combination chemotherapy using AG3340 and either Taxol or carboplatin.

Conclusion

The role of matrix metalloproteinases in cancer has proven to be significant, taking part in angiogenesis, growth, metastasis, and apoptosis. The complexity of the MMPs and their endogenous and exogenous inhibitors leaves a great deal to be discovered. Improved understanding of the sources, target, and mechanisms of action of both MMPs and their inhibitors is essential for effective and safe treatment. Clinical studies, however, are promising and may lead to a novel, noncytotoxic treatment to be used in combination therapy. *(Mr. Filsinger is a medical student at the State University of New York at Buffalo.)* ❖

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Special Feature

Novel High-Dose Chemotherapy Regimens for the Treatment of Advanced Cancer: Part II

By Daniel M. Sullivan, MD, and James S. Partyka, PharmD

Mechanisms of Resistance to Topo I and Topo II Inhibitors

Preclinical studies in our laboratory and others have defined several mechanisms of resistance to topo I and II inhibitors.[1-4] This includes resistance to VP-16 and TPT, drugs we currently use in high-dose chemotherapy regimens for MM, NHL, ovarian cancer and other refractory malignancies. Drug resistance to topo II poisons may result from: 1) altered transport of the drug due to overexpression of P-glycoprotein (Pgp), multidrug resistance-associated protein (MRP), or lung resistance-related protein (LRP); 2) a mutation in the gene for topo IIα such that an enzyme with altered DNA cleavage activity is expressed;[5,6] 3) an attenuation of nuclear topo II content;[7,8] and 4) an altered subcellular distribution of topo IIα such that it is no longer associated with the nuclear matrix[9,10] or, due to a loss of its nuclear localization signal, it remains in the cytoplasm.[11,12] Although the majority of studies have focused on the role of topo IIα in drug resistance, recent in vitro experiments have shown that changes in topo IIβ may also be involved in drug resistance,[13,14] and that the β isoform may be the preferred target of daunomycin, mitoxantrone and/or m-AMSA.[13,15] The mechanisms of resistance to topo I inhibitors defined in cell lines generally parallel those of topo II with respect to a down-regulation of topo I content[16,17] and inactivating mutations.[18] Altered drug transport due to the overexpression of Pgp results in minimal levels of resistance to TPT[19] and CPT-11/SN-38,[20] while resistance to TPT[21] (but not to CPT-11) may involve increased levels of MRP. A novel finding with topo I inhibitors, specifically CPT, is that the drug may be involved in regulating its own sensitivity. A continuous exposure of human KB cells to CPT has been shown to decrease topo I protein levels, decrease protein-linked DNA strand breaks, and decrease CPT cytotoxicity.[22] The CPT-induced degradation of topo I is likely due to ubiquitination of the topo I population involved in a complex with DNA, which is subsequently proteolyzed by the 26S proteasome.[23] TPT has also been shown to stimulate ubiquitin-mediated destruction of topo I.[24] Except for AML,[25] the role of alteration of topo I and/or topo II in clinical drug resistance is largely unknown.[26]

Clinical Studies with High-Dose Topotecan

Recently, Donato and colleagues investigated the use of escalating doses of topotecan in combination with cyclophosphamide (3 g/m^2, total dose) and melphalan (140 mg/m^2, total dose) followed by stem cell rescue in 22 patients (median age 45 years) with advanced ovarian cancer.[27] Of the 22 patients, six patients were chemoresistant, seven patients were chemosensitive in relapse, seven patients had evidence of disease upon a second look laparotomy, and two patients were in complete remission. Topotecan was escalated from 6.25 mg/m^2 to 13.75 mg/m^2 (total

Figure 1

Overall survival of refractory cancer patients treated with high-dose TIME followed by stem cell rescue

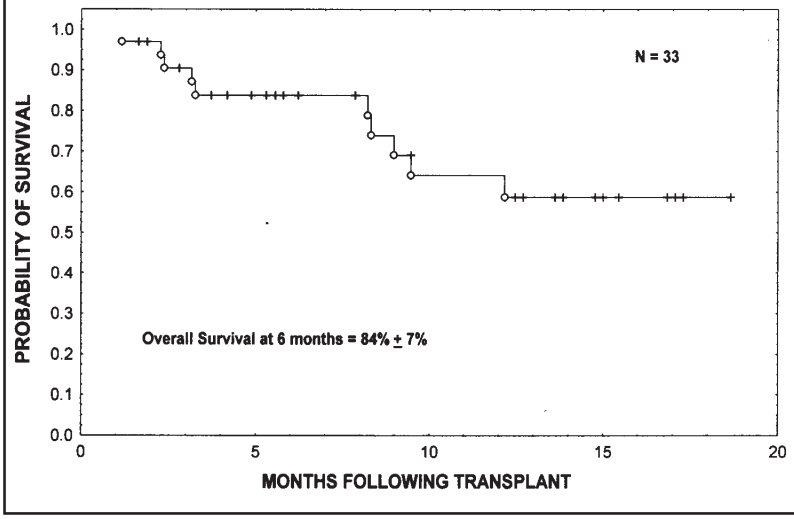
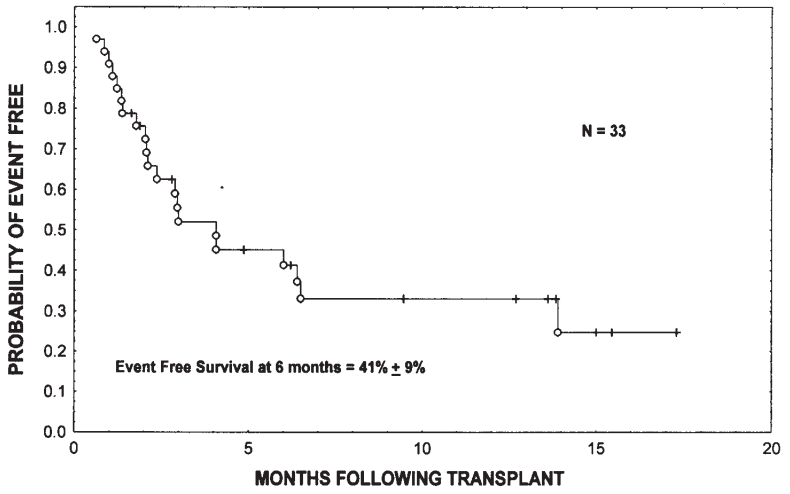


Figure 2

Event Free Survival of refractory cancer patients treated with high-dose TIME followed by stem cell rescue



dose) administered over five days from day-6 to day-2. Patients received peripheral blood stem cells on day 0 followed by G-CSF. Regimen-related toxicity was limited to grade 2 mucositis (n = 6) and grade 2 diarrhea (n = 2). The median day to engraftment was day +9 for an ANC greater than 500/uL and day +14 for platelets greater than 50,000/uL. Of interest, the overall response rate for patients with measurable disease was 91.7% (76% CR, 19% PR, 5% SD). The investigators concluded that although the MTD of topotecan had not been reached, the regimen was active in the treatment of advanced ovarian cancer.

Currently, our institution is completing a phase I/II high-dose topotecan study in combination with etoposide and ifosfamide.[28] Topotecan has been dose-escalated from 10 mg/m^2 to 64+ mg/m^2 (total dose over 3 days), with stem cell rescue, in combination with ifosfamide and etoposide at fixed total doses of 10 g/m^2 and 1500 mg/m^2, respectively. A two-hour infusion of ifosfamide is followed immediately by a 30-minute infusion of topotecan on days -8, -7, and -6. On days -5, -4, and -3, a continuous infusion of 500 mg/m^2/d etoposide was administered. We have accrued 38 patients (25 with breast cancer, 6 with NHL, 6 with ovarian cancer, 1 with testicular cancer) to this study. The maximum tolerated dose of topotecan has not been reached, although grade 3 or 4 mucositis was observed in 97% of patients. Other grade 3 or 4 regimen-related toxicities include enteritis (42%), nausea and vomiting (37%), and hematuria (8%). Since publication, there were two treatment-related deaths. One patient died on day +10 due to sepsis and cardiac failure and the other patient died on day +34 due to sepsis and CNS *Aspergillus*. The median day to engraftment is day +10 for an ANC greater than 500/uL and day +16 for platelets greater than 50,000/uL, untransfused. Thus, topotecan can be given in high doses in combination with an alkylating agent and a topoisomerase II inhibitor. Complete (4) and partial (9) responses have occurred in 38 patients (34%), while stable disease has been observed in 14 additional patients. The overall response rate in refractory NHL and refractory metastatic breast cancer was 67% and 31%, respectively. The overall survival and event free survival (EFS) for all patients at six months were 84 ± 7% and 41 ± 9%, respectively. *(See Figures 1 and 2)*. RT-PCR analysis and Western analysis of patient peripheral blood lymphocytes after topotecan administration generally demonstrate a significant decrease in topo I protein levels (more than mRNA levels) and an increase in topo IIa mRNA expression. In summary, our experience at this institution suggests that high-dose topotecan in combination with ifosfamide and etoposide can result in response rates and prolonged EFS in patients with refractory cancer.

Conclusion

Determining the optimal dose and sequencing of agents in the high-dose setting is essential in the treatment of various malignancies. Recently, published data from phase II trials utilizing high-dose topotecan appear to be promising, although current studies are limited by small sample sizes and short patient follow-up. Further investigation is warranted. Therefore, at our institution we are continuing to develop phase II trials utilizing high-dose topotecan in both chemosensitive lymphoma and chemosensitive metastatic breast cancer patients. *(Dr. Sullivan is Associate Professor of Medicine and Biochemistry & Molecular Biology, H. Lee Moffitt Cancer Center and Research Institute at the University of South Florida, Division of Blood and Marrow Transplantation; and Dr. Partyka is Assistant Professor of Medicine, H. Lee Moffitt Cancer Center and Research Institute at the University of South Florida, Division of Blood and Marrow Transplantation.)* ❖

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Funding News

The National Science Foundation Division of Molecular and Cellular Biosciences (www.nsf.gov). The National Science Foundation's (NSF's) **Division of Molecular and Cellular Biosciences (MCB)** supports research and related activities that contribute to a fundamental understanding of biological processes at the molecular, subcellular, and cellular levels. Investigator-initiated research proposals are considered in the following programs: biomolecular structure and function, biomolecular processes, cell biology, and genetics. Programs in MCB also support fundamental studies leading to technological innovation, proposals with substantial computational components, and multidisciplinary and small group research. Biodiversity and biotechnology are major focal points of MCB. MCB programs particularly encourage submission of proposals involving microbial biology, plant biology, theoretical/computational aspects of molecular and cellular studies, molecular evolution, and biomolecular materials. Genomic approaches are encouraged in all areas. In fiscal year 1999 the division coordinated a special BIO-wide competition for Microbial Observatories. A new program announcement for this activity is under development and will soon be available at the NSF Web site. The NSF MCB also considers proposals for limited support of special meetings and workshops. NSF MCB program areas and grant target dates are as follows:

• **Biochemistry of Gene Expression** supports research using biochemical and molecular biological methods to investigate mechanisms for the replication, expression, transfer, and stability of genetic information—both DNA and RNA. These studies primarily involve in vitro biochemical approaches. Gene expression mechanisms are a major focus, including transcription and processing of mRNA regulatory features, chromatin architecture, RNA stability, and translational mechanisms. Other areas of focus include DNA replication, mutation, and repair. *(Target Date: Jan. 10, 2000.)*

• **The Biomolecular Processes** cluster considers projects on molecular mechanisms by which genetic and metabolic processes occur in plant, animal, and microbial organisms. These processes and related regulatory features are the areas of emphasis. Review of research is organized around the themes of biochemistry of gene expression and metabolic biochemistry. *(Target Date: July 10, 2000.)*

• **The Biomolecular Structure and Function** cluster focuses on understanding the structure and function of biological macromolecules, including proteins, nucleic acids, polysaccharides, and lipid assemblies. The research supported by this cluster encompasses a broad range of topics and techniques. The cluster encourages multidisciplinary and innovative efforts at the interfaces of biology with physics, chemistry, mathematics, and computer science. The organized areas for review are molecular biochemistry and molecular biophysics. *(Target Date: Jan. 10, 2000.)*

• **The Cell Biology** cluster funds research on the

structure, function, and regulation of plant, animal, and microbial cells. Review of research is organized around the themes of cellular organization and signal transduction. *(Target Date: July 10, 2000.)*

• **Cellular Organization** supports studies of the structure, function, and assembly of cellular elements such as the cytoskeleton, membranes, organelles, intracellular compartments, intranuclear structures, and the extracellular matrix (including cell walls). This encompasses structural and dynamic aspects of cellular and intracellular motility, meiosis and mitosis, and cell shape and polarity, including the mechanisms of endocytosis, exocytosis, and intracellular trafficking of membranes and macromolecules. (*Target Dates: Jan. 10 and July 10, 2000.)*

• **The Genetics** cluster considers a wide range of studies directed toward answering significant questions of organization, recombination, function, regulation of function, and transmission of heritable information in all organisms from viruses and microorganisms to plants and animals. Specific areas include, but are not limited to, mechanisms of gene regulation, chromosome structure and replication, epigenetic phenomena, DNA repair and recombination, sex determination, genetic interactions between genomes, and molecular evolution. The methodologies used should be appropriate to the questions asked about genetic structure or/and function. Interdisciplinary proposals or proposals asking genetic questions but using methodology from other scientific disciplines will be co-reviewed in a manner that will ensure effective and fair evaluation of each proposal. *(Target Date: July 10, 2000.)*

• **Metabolic biochemistry** supports research on many aspects of the dynamic activities of cells. This includes characterization of the biochemical pathways and other processes by which all organisms acquire, transform and utilize energy from substrates and synthesize new small molecules and macromolecular cell components. The diversity of primary and secondary metabolism and mechanisms of metabolic regulation, in response to both internal and external signals, are major topics of interest. Also included are biotransformations of environmentally significant compounds; manipulations of metabolism with practical applications; quantitative and temporal aspects of metabolism; integration and subcellular organization of metabolic processes; and use of new methods and technologies to conduct metabolic studies. *(Target Dates: Jan. 10, and July 10, 2000.)*

• **Molecular biochemistry** emphasizes the correlation of function with the known structure of biological macromolecules and supramolecular structures, e.g., multienzyme complexes, membranes, and viruses. Additional areas of responsibility include: ribosomal function; the mechanism and regulation of enzyme and RNA catalysis; biochemical reactions involved in bioenergetic processes and photosynthesis; key biochemical processes involved in protein synthesis and folding; and the synthesis of biomolecular materials. Approaches typically include combinations of biochemical, molecular biological, chemical, physical, as well as genetic techniques applied in an integrative manner to address the above topics. *(Target Dates: Jan. 10, and July 10, 2000.)*

• **Molecular Biophysics** supports research on the structure, dynamics, and interactions of biological macromolecules. This includes the determination and study of the three-dimensional structure of macromolecules; assembly and architecture of supramolecular structures (e.g., multienzyme units, viruses, membranes and contractile proteins); energy transduction; structure and dynamics of photosynthetic reaction centers; and mechanisms of electron and proton transfer in biological systems. Typical methodologies include: theory and computation; x-ray diffraction; magnetic resonance; optical spectroscopy; specialized microscopy, such as atomic force; and mass spectrometry. (*Target Dates: Jan. 10, and July 10, 2000.)* ❖

CME Questions

1. Dencdritic cells:
   a. require helper $CD4^+$ cells to stimulate the production of cytotoxic T cells.
   b. require monocytes to to stimulate the production of cytotoxic T cells.
   c. can stimulate 10-100 T cells.
   d. can stimulate 100-3000 T cells.

2. Drug resistance to topoisomerase II poisons may result from all of the following *except:*
   a. altered drug transport.
   b. mutation of the topo II gene.
   c. increased nuclear topo II content.
   d. altered subcellular topo II distribution.

3. Radiofrequency ablation:
   a. uses low frequency wave to mechanically disrupt tissue.
   b. uses high frequency alternating current to heat tissue.
   c. uses high frequency alternating current to freeze tissue.
   d. is not limited by the size of the tumor.

4. The irreversible inhibition of the active site of metalloproteinases by TIMPS is provided by their:
   a. N-terminal domain.
   b. C-terminal domain.
   c. beta pleated sheet.
   d. hydrophobic domain.

In Future Issues: Inhibition of p53-Dependent Apoptosis