RESEARCH ARTICLE

Cryoablation of Prostate- Improving Efficacy and Safety

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Abstract

Cryoablation has been shown to be an effective therapy for all stages of prostate cancer. Though judged efficacious, complications of treatment and persistent disease, although minimal, show the need for improvement. To this end, research has focused on new technology design for cryosurgical apparatus and imaging techniques. Adjunctive therapy, focusing on increasing cell death by apoptosis, also plays a role in this new direction with the intent of increasing cell death in the frozen tissue. Additionally, incorporating methods of protecting adjacent structures, including the urethra, bladder neck sphincter, urogenital diaphragm, neurovascular bundles, and rectum, are critical to achieving a successful outcome and continue to evolve. Several strategies to protect these structures are now commonplace as part of a cryosurgical procedure. These strategies include real-time temperature monitoring, visualization of ice growth during freezing, active heating and even the injection of protective media have emerged as methods to protect these structures. Another more recent procedural application is partial gland ablation or image-targeted focal therapy, developed to maintain cancer control yet minimize the risk of collateral damage to the various structures. Each of these methods has been shown *in vitro*, *in vivo*, and clinically to be beneficial. This article describes the directions that cryoablation has taken in an effort to improve procedural efficacy while reducing/eliminating associated co-morbidities.

Key Words: Cryosurgery, Focal Therapy, Co-morbidity, Temperature Monitoring, Nerve Sparing, apoptosis, cryotherapy, neuroprotection, cryosurgery, focal therapy, selective protection

Introduction

Since its first use in the mid 1960's, cryosurgery of the prostate has developed in stages. Almost abandoned early in the 1980's, technological advances over the past 25 years have led to a resurgence in the utilization of cryoablation as a primary therapeutic option for cancer of the prostate. The attractiveness of modern cryoablation is related to numerous factors, including a global acceptance of minimally invasive therapies, rapid adoption of intra-operative ultrasound and other imaging techniques, advances in cryosurgical device technology, the ability to provide transient, selective protection to adjacent, non-targeted tissues and the prospects of effective adjunctive therapy. Cohen, et al.[1] reported the first ten-year retrospective series on patient outcome which yielded an impressive 77% biopsy negative result averaged over all grades and stages of prostate cancer. Following this report, the American Urological Association published a Best Practices Policy Statement[2] that identified cryoablation of the prostate as both primary and salvage treatment options in 2008. Studies over the last ~15 years have detailed the efficacy and benefit of cryosurgery and compared the results favorably to other therapeutic options.[3-12] Cryoablation of the prostate continues to evolve as a primary treatment for cancer. The published literature suggests that cryoablation has a similar 5 and 10 year outcome (biopsy and biochemical disease free survival (BDFS)) for low and moderate risk disease and improved outcome for high risk disease compared to other therapies, including radical prostatectomy, radiation (conformal and external beam) and brachytherapy.[10-18] Examining co-morbidities in these and other studies, cryotherapy has been found to have a lower overall rate of rectal injury and incontinence and a similar rate of impotence compared to radical prostatectomy, brachytherapy of radiation therapy.[1-18] The present day technique of treatment focuses on improving the efficacy of targeted freezing and protection of the adjacent extra-prostatic tissues. Nevertheless, the rate of persistent disease, which is in the range of 10 to 25% depending upon the stage, and continued concern with co-morbidity, including damage to the urethra, incontinence, loss of potency, etc., clearly shows the need for continued improvements in the technique.[11,19-21]

Increasing Tissue Destruction

The technological problem in efficacious tissue freezing is related to the characteristics of the freeze-thaw cycles used in cryoablation. Two major facets, each with subdivisions, are involved in the effort to improve efficacy. The first of these is the process of freezing. To destroy cancer tissue with certainty, cryoablation typically requires exposure of the prostatic tumor mass to approximately -40°C with a repetitive freeze-thaw cycle.[22-26] Attaining this temperature at the margins of the tumor requires extending the leading edge of the ice ball several millimeters beyond the tumor edge (positive freeze margin). When freezing the prostate, the ice front is allowed to grow beyond the boundary of the prostatic capsule. While necessary, with the application of a positive freeze margin a substantial volume of non-targeted tissue is partially damaged following exposure to temperatures ranging between -1°C and -40°C.[27-30] For example, if a 3 cm diameter anatomical target was treated with a 1cm

"over-freeze", as is commonly applied in freezing tumors of the liver or kidney, approximately 75% of the total frozen mass would be contained within the positive freeze margin which would consist of non-targeted tissue. A 5mm or 2.5mm positive freeze margin results in ~55% and 37% of the total frozen mass contained within this partially lethal zone, respectively. The necessity of a positive freeze margin coupled with the anatomical location of the prostate results in a number of considerations to avoid complications due to injury to adjacent tissues. Intraoperative ultrasound permits physicians to visualize ice front progression and stop freezing prior to the ice front penetrating non-target tissues such as the rectal wall and urinary sphincter. While offering a potential solution to reducing collateral tissue damage, limiting ice growth to protect normal tissues may result in the incomplete ablation of peripheral tumor tissue, leaving the significant possibility of disease persistence or recurrence.[2,5,10,11] To this end, several studies have focused on the development of new devices to deliver ultracold ablative temperatures deep into tissue reducing the distance between the ice ball edge and the -40°C isotherm.[27,28,31] The ability to drive the -20°C and -40°C isotherms closer to the edge of the ice ball would enable a reduction of the size of the positive freeze margin thereby potentially reducing collateral damage. While these devices remain in the investigational stage, these studies none-the-less demonstrate the potential benefit of these new systems.

Another approach to improving procedural efficacy is through the use of adjunctive therapy to increase the destruction of cells in the periphery of the frozen volume where the tissue temperature is in the range of 0 to -40°C.[32] The object is to make the ablation more closely correspond to the volume of tissue frozen, as shown by the imaging apparatus, thereby reducing the need for a positive freeze margin while assuring cancer destruction to the edge of the ice ball. Considerable research has focused on the use of adjunctive agents to increase the efficacy of freezing by their effect on the cells in the periphery of the frozen volume.[33-36] For this purpose, cytotoxic drugs, antifreeze proteins, chemical agents, immunologic enhancing drugs, TNF alpha, and others have been used.[37-52] Irradiation has also been utilized. The timing for use of these agents in relation to the cryoablative procedure is not well established, but all contribute to cell death by apoptosis.[20,21,32,35,36] While an ongoing area of research, several studies have shown promise in elevating the minimal lethal temperature for prostate cancer from -40°C to approaching -10°C in *in vitro* and murine models using calcitriol pre-treatment.[48-52] More recent studies have focused on the combination of immunotherapy and cryoablation to improve cancer destruction, both localized and metastatic disease.[44,53-55] Regardless of the agent utilized, the goal of these efforts is to "make ice lethal at 0°C" while eliciting minimal side effects.

Target Protection of Anatomical Structures

With regard to safety, one challenge in prostate cryoablation relates to the risk of damaging juxtaposed, non-targeted anatomical structures including the urethra, rectum, urogenitial diaphragm/external urinary sphincter, bladder neck smooth muscle

sphincter and paired neurovascular bundles. During the course of a cryoablative procedure, care must be taken to avoid damaging these anatomical structures in order to prevent function-related complications (i.e. incontinence and impotence). In practice, a series of protective strategies are employed, with others under investigation, to provide the transient (few minutes) protection necessary during freezing to avoid the creation of co-morbid conditions. These strategies include control of the ice by imaging, application of heat, introduction of protective compounds and utilization of buffering media to name but a few.[56-59] Each of these strategies has been implemented to protect these structures with varying degrees of success.

The Rectum

Damage to the rectum, resulting in fistula formation was a significant complication during early years of prostatic cryosurgery. In modern times the application of both ultrasound and standard protective strategies has now made the occurrence of rectal fistula rare in clinical practice. Techniques that are commonly employed to prevent damage include ultrasound-based ice front monitoring, mechanical deflection of the rectal wall away from the prostate, and injecting protective media. When a downward pressure is applied to the transrectal ultrasound probe, an expansion of Denonvillier's fascia results along with rectal wall displacement allowing a 5-6mm zone into which ice can grow without damaging the rectal wall. By widening the space between the posterior prostatic capsule and the anterior rectal wall, the ice ball is allowed to encompass the prostate in a colder isotherm, e.g. ,-20°C, which is located deeper than ~3-5mm inside the ice edge with today's Argon-based cryoablation devices.[27-30] A second technique commonly utilized relies on the injection of a freeze "buffering" medium, such as isotonic saline or dextrose-saline solution, into Denonvillier's fascia.[56,57] This solution injection contributes to both the expansion of the space and a retardation of ice front growth in the event that the freeze zone progresses into this region. The retardation of ice growth is afforded as a consequence of both protective agents (sugars, buffers, ions, agents, etc.) in the solutions that lower the solution freezing point as well as the release of latent heat of crystallization upon ice formation. That is, for water to undergo a phase change from a liquid to a solid crystal, the water molecules release heat energy as the crystals form. During this process, the tissue mass that is freezing remains at approximately -0.6°C, the melting point of saline solution. Since the duration of protection necessary is in the order of minutes at the freeze zone margin, a modest bolus of media (50-100 ml) provides adequate transient protection. However, the problem with using saline to widen Denonvillier's space is the need for a constant injection of fluid, as the tissues quickly absorb the solution in short order.

Mouraviev *et al*.[60] reported on a series of pre-clinical studies that involved a custom formulated protective solution with putative protective actions beyond that of latent heat release. Solution formulation included 5% DMSO, membrane stabilizing hydrocortisone, and the protease inhibitor contrycal. No rectal fistulae were observed following freezing. Other methods of protecting the rectum by instrumentation have been suggested.

Bischoff and his colleagues have designed an insulating probe made of silicone which is placed in Denonvillier's fascia prior to freezing.[61] In clinical practice, Cytron *et al.* reported a technique whereby two ultrathin cryoprobes are placed between the prostate and rectal wall for active warming during prostate freezing.[62] This effectively prevented rectal injury.

The Urethra

The Urethra traverses the central volume of the prostate gland. In early attempts at prostate cryoablation in the 1960-1970's, the urethra was fully surrounded by ice resulting in extensive post-thaw sloughing of the urethral wall and obstruction of the urethral lumen. To avoid this undesirable quality of life complication, the cells of the urethral lining are now routinely protected by active warming techniques through the insertion of a warming catheter that expands under fluid pressure to conform to the luminal channel.[58,63-65] The catheter is designed as a counter current heat exchanger operating at 38 to 40°C to provide sufficient warming and protection of a 1-2mm thickness of the urethral wall. The reported incidence of urethral sloughing following cryoablative procedures with use of a warming catheter ranges between 0 to 15% compared to rates in excess of 25% without the catheter.[7,66] It is not common for prostatic cancer cells to be located in close proximity to the urethra, particularly in the primary setting, but this may be more of a concern in relation to persistent disease in the salvage setting.[67] Too warm a temperature in the protective catheter will reduce the incidence of urethral sloughing and resultant incontinence or obstruction, but will permit the survival of cancer cells adjacent to the urethra.[68] Nevertheless, with current techniques, the incidence of urethral sloughing has been reduced substantially.[1,2,19]

The Continence Structures: Bladder Neck Sphincter and Urogenital Diaphragm

The flow of urine from the bladder is regulated by two muscular structures, the bladder neck sphincter (smooth muscle sphincter) and urogenital diaphragm (striated urethral sphincter), both requiring transient protection during cryosurgery to assure continued continence. Protection of the bladder neck intrinsic sphincter is often provided with the aid of a bolus of saline injected into the bladder or by residual urine. The heat content of these liquids minimizes the progression of the ice ball into the sphincter by retarding ice growth. As with the urethra, other strategies of protection have been employed including the utilization of protective media[59] and active heating.[58,63,64] Each of these approaches has been shown to provide an effective protective strategy for the bladder neck region. From a practical point of view, it is difficult to injure the bladder neck unless a probe went astray and was inadvertently placed too deep into the bladder neck. In addition to the intrinsic bladder neck sphincter, protection of the urogenital diaphragm is also critical to the retention of continence following cryoablation. Another area that is at risk and that is routinely protected using the "heat sink" effect is the trigone area of the bladder and the mucosal lining of the bladder neck. As the base of the prostate lies directly adjacent to the trigone area of the bladder neck and is relatively can be damaged with improper probe placement or over freezing. Damage to this area is rare, but

has been noted in a few cases. This area is typically protected by leaving a moderate volume of irrigation fluid/urine in the bladder prior to placing the urethral warmer. This "volume" of body temperature liquid keeps the bladder neck and trigone from freezing, even if a probe is very close. Unlike the bladder, the urogenital diaphragm, located distal to the prostate, lacks a comparable heat source to prevent collateral damage. Most commonly, protection of the urogenital diaphragm is assured through active temperature monitoring using thermocouples as well as through ice front visualization under intraoperative ultrasound. Protection of this structure has been accomplished in the past by injection of a special solution to minimize ice progression into this area.[60] In general, we don't clinically see urinary incontinence after primary therapy as there is good blood supply to the sphincter and probes should be properly positioned under real-time ultrasonography. In contrast, the external sphincter is more susceptible to injury due to decreased vascular perfusion following radiation due to obliterative endarteritis. With the standard application of one of the various protective options during cryoablation of the prostate, post procedural incontinence rates have been drastically reduced over the last 15 years.

Neurovascular Bundles

Due to the anatomical location of the neurovascular bundles (NVBs) just outside the capsule of the prostate, coupled with aggressive freezing and positive freeze margin employed to assure cancer eradication, nerve damage can result in a higher likelihood of impotence associate with cryoablation of the prostate. As such, much attention has been given to protection of the NVBs. The NVBs are parasympathetic post-synaptic ganglia located on the posteriolateral aspects of the prostatic capsule that control erectile function. Nerve fibers within the ganglia and the tracts that supply the bundles are sensitive to freezing.[69,70] Experience has shown that 40-55% of patients treated by prostate cryosurgery experience either temporary or permanent damage to these nervous structures.[1,2,7] While a significant number of patients may experience full or partial recovery in the twelve months following therapy, the balance of patients are left to seek alternative therapies aimed at restoration of potency.

Since these structures are located in an area that is encompassed by the far margin of the freeze zone, only brief, transient protection is necessary. One method of protection that has been examined is the use of active heating through the placement of a cryoneedle outside the capsule operated in a heating mode in an effort to create a "divot" of warming indentation in the advancing freeze front.[65] This approach lacks precision as it is sometimes difficult to clearly recognize the position bundles thereby compromising effective cryoneedle placement. For instance, the NVBs follow a curvilinear path but the cryoprobe is straight, thereby preventing coaptation and protection along the entire length of the NVB. The exact temperature can be defined with the aid of a thermocouple inserted into an area of the NVB.[72] In principal, both NVBs can be locally protected especially using whole gland cryoablation. This method has been explored at a number of prostate centers with varying degrees of success. Issues related to challenges with probe placement and technique reliability are often associated with

this approach. Another approach employs a selective neuroprotection strategy that includes the use of liquid cocktails as injectables (i.e. saline, preservation agents, etc.) into this region with the purpose of providing a few minutes of protection during the terminal phase of the freeze cycle.[73,74] These approaches have included the use of agents including dimethyl sulfoxide, glycerol, alcohol, free radical scavengers, protease inhibitors, growth factors, etc. The mechanism of action of this strategy appears to be less related to the composition of the injected liquid solution and more to the release of latent heat during the freezing process. While latent heat release plays a dominant role in this neuroprotective strategy, Mouraviev *et al*.[74] reported on the use of a custom formulated protective solution with putative protective actions beyond that of latent heat release. This protective formulation strategy includes ice control combined with anti-stress and membrane stabilizing properties and was demonstrated to be modestly effective. The protection solution contained DMSO as well as an antibiotic, a protease inhibitor and an antifreeze protein isolated from the winter flounder *Pseudopleuranectes americanus*. The benefit of the use of various cryoprotective solutions remains investigational.

Focal Therapy

One strategy that recently has been garnering attention is that of image-targeted focal ablation (focal therapy) of the prostate whereby one or both neurovascular bundles are spared from exposure to freezing altogether.[75-81] Focal therapy ablates only the portion of the prostate where a cancerous tumor(s) is located. While rapidly growing in clinical practice, less than 10 years ago focal therapy was considered experimental and of high risk by many physicians. Today, studies are demonstrating focal therapy can be an effective treatment option for the well-selected patient with non-metastatic disease demonstrating short-term outcomes comparable with that of radical prostatectomy. Focal therapy has also been shown to be effective as a salvage option for radiation and ablative therapy failures. The promise of focal therapy using cryoablation, as well as other energy sources including HiFu, RFA, Laser, etc., was recently highlighted in a series of presentations at the International Symposium on Focal Therapy and Imaging in Prostate and Kidney Cancer 2020.[82-87] These presentations discussed the application, outcomes and benefits of focal therapy procedures which included significantly reducing the risk of damage to adjacent tissue structures. While highly promising, long term prospective study outcomes have yet to be reported.

Summary

Cancer of the prostate gland has several treatment options. No one method of treatment appears optimal for all patients with this disease because of diverse factors which affect the therapeutic outcome. Cryoablation has proven competitive with alternate methods of therapy in all stages of cancer, including salvage after irradiation.[1,6,7-10,19,22] Nevertheless, the incidence of persistent disease and complications attendant to freezing compel a search for improvement in technique.

Current efforts focus on two major strategies, 1) the need for more effective controlled freezing and 2) the need for improved protection of the normal tissues in

close proximity to the prostate gland. More effective freezing requires that the lethal effect on the cells must more closely correspond to the frozen volume as seen on the monitored image. Improvements in cryogenic apparatus, likely in the direction of nitrogen-cooled probes[28], are needed. The integration of cryoablative neoadjunctive therapy to increase the lethality of mild freezing temperatures is a critical need which would eradicate the cancer cells in the range of 0-40°C.[32] This would reduce the necessary positive freeze margin and spare the extra-prostatic tissue. The critical discovery that adjunctive therapy enhances death by apoptosis caused by freezing is a clear direction for further research.

The clinical utility of using protective strategies to mitigate the unwanted effects of freezing on the neurovascular tissues, rectum, and urethra have become common practice in cryoablative technology today. Further investigation on this subject, perhaps especially on transient protection of the neurovascular structures, is needed. Freezing only an area of the prostate (focal ablation), and avoiding total prostatic cryoablation, is a growing clinical practice[77-87] and has shown tremendous progress but requires long term follow-up to fully judge merit.

In this report, we have defined current problems and issues in cryoablation of the prostate and some things/manuevers that can be done to improve therapeutic outcome. In general, the technique continues to evolve as new technologies, approaches and techniques are developed. The goal of these efforts is to develop methods of improving efficacy, maximizing cell death in the prostate, while avoiding damage to adjacent normal tissues.

Declarations

Funding: This study was supported in part by funding from the National Institutes of Health – National Cancer Institute 1R43CA210761-01A1 awarded to CPSI Biotech.

Conflicts of Interests: JMB and RVB are employees of CPSI Biotech. JGB and TP have no competing interests.

Authors' Contributions: JMB and JGB prepared the draft manuscript. JMB, RVB, TP and JGB provided review and revision input for the manuscript. All authors read and approved the final manuscript.

Availability of Data and Material: The data that support the findings of this study are available from CPSI Biotech but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of CPSI Biotech.

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