Principles of Cryoablation

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Abstract

This chapter describes the development of the use of freezing temperatures in therapy. The principles of biological freezing were established in early work on frostbite and on cryopreservation protection. These led to an understanding of the tissue response to freezing, the mechanism of cryogenic injury, and the techniques of cryosurgery. Modern cryosurgery requires monitoring by temperature measurement and by diverse imaging techniques, which continue to evolve.

Keywords

Cryosurgery • Cryotherapy • Cryoablation • Adjunctive therapy • Tissue freezing • Tissue ice

Introduction

The use of low temperature to palliate pain and to manage inflammation has been exploited since the dawn of history. The written records of the Egyptian surgeon Imhotep dating back to 2600 BC describe the therapeutic use of cold [1]. The first use of freezing as a debulking and potentially curative process was extensively described by Arnott in the mid-1800s following the use of "salted ice" mixtures (~ −24 °C) to treat visible tumors of the breast and uterus [2]. Half a century later stepwise advancements in cryogenic engineering would permit access for medical use to ultracold cryogens. Key developments included the discovery of the Joule-Thomson effect in 1853, cryogen liquefaction (Caillete 1877; von Linde 1895) and Dewar's 1892 invention of the vacuum insulated thermos (dewar) essential to maintaining and handling a volume of liquefied gas.

Liquid cryogens found their earliest therapeutic use at the turn of the twentieth century when White reported on the successful treatment of

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various dermatologic conditions [3, 4]. Over the next half-century numerous cryogens were employed including liquid CO_2, N_2O, liquid air, liquid oxygen and ethers. LN was first employed in 1950 as a non-combustible cryogen to replace liquid oxygen [5]. To this point in time dermatologic applications of freezing were limited to surface treatments with cryogen sprays or topical liquid application. In 1961, Cooper and Lee [6] developed the first cryoprobe that could be inserted through the skin for treatment of bulky skin lesions and or visceral tumors. With this development dermatologists had access to a multiplicity of cryosurgical tools supportive of relatively precise tumor treatment.

Principles of Biological Freezing

With the growing interest in diverse cryoablative strategies, a need to understand underlying principles of freezing and its consequential mechanisms of action in tissue became apparent. Numerous studies of the damaging effects related to frostbite along with a developing understanding of cellular freeze protection during cryopreservation procedures established a base line of relevant knowledge.

The application of a cryogen in various forms (i.e. metallic probe, fibrous wick, surface spray, etc.) to a targeted tissue, once "activated," acts as a heat sink to remove thermal (heat) energy. As tissue cooling progresses, water molecules slow, tend to aggregate into a structured lattice and form an ice crystal. Ice growth proceeds outwardly from the "cryoprobe" by accretion of water ahead of the freeze front at a rate dependent on the heat extraction capabilities of the cryogen. The rate of freezing is always more rapid proximal to the "cryoprobe." Hence, the rate of freezing varies over the radius of the freeze zone resulting in less damaging effects in the periphery of the frozen tissue mass. This discontinuity may yield cell survival within the distal regions of the tissue target or in those cells near active vasculature. For this reason, a second freeze following the first thaw is common practice since a second "partially lethal" freeze yields an additive destructive outcome. The second freeze, while use has been historically empirical, gains significance as it is now recognized that indolent cancer cells and cancer stem cells are far more resistant to varied therapeutic assaults and are no doubt the foci of cancer recurrence [7].

Tissue Response to Freezing

Depending upon the severity of the freezing during a cryosurgical procedure, the tissue's responses to cold injury may range from reversible inflammation to cellular destruction. This difference is the basis for a selective therapeutic response. Short duration freezing at elevated subzero temperatures produces only a mild inflammatory response with limited therapeutic uses such as the treatment of retinal detachment. Severe freezing produces destruction of cells through two processes: (1) physical effects of cell rupture due to osmotic shock and intracellular ice formation and (2) activation of stress signaling cascades that launch numerous molecular mechanisms of cell death (i.e. apoptosis, autophagy and necrosis). Some differences in the sensitivity of diverse cell types to cold injury and even freezing have been reported which may be exploited for therapeutic purpose [8].

The cryogenic lesion is characterized by a central portion of coagulation necrosis, which collectively consists of death via physical trauma, rapid-onset apoptosis, and necrotic populations. With a relatively thin peripheral zone or freeze margin cell destruction is uncertain. Shortly after thawing, the tissue appears hyperemic within the border region of the previously frozen volume with an edematous central zone. Maximal levels of apoptosis are evident within the core within 1–2 h following thawing, whereas elevated levels are seen in the periphery several hours later while necrosis is observed immediately post-thaw (primary necrosis) and in the following days (secondary necrosis). The border of the previously frozen tissue may be critical to therapeutic management. In this region tissue temperatures ranged from 0 to −20 °C, yielding some live cells, some dead, and others partially damaged

hovering between life and death [9, 10]. It is within this region that high numbers of delayed apoptotic and secondary necrotic cells are evident. Hence, the therapeutic challenge is to ensure the death of all cells in this region. While a challenge, the involvement of molecular mechanisms of cell death offers the potential for combination strategies to enhance death [7].

The injured tissue begins the repair process quickly with the infiltration of inflammatory cells migrating through the necrotic tissue. Over the following weeks to months, a fibrous, pliable collagen scar laid down by fibroblasts slowly replaces the necrotic tissue The preservation of the collagenous matrix helps retain the tissue architecture which facilitates tissue repair, and healing.

Mechanisms of Cryogenic Injury

The mechanism of tissue injury from freezing is complex as the numerous consequences of a freeze-thaw cycle have a global impact on cellular homeostasis. Direct injury to the cells caused by ice crystal formation might also include microcirculatory failure. The cascade is completed with the post-thaw induction of apoptosis and cellular necrosis. Extracellular ice crystal formation, especially in the peripheral region of the freeze zone, removes water from the cells causing major deleterious metabolic disequilibria related to solute concentration, the "solution effects". Ice crystals also cause mechanical damage due to cell membrane disruption, intracellular ice crystal formation and shearing forces, especially in highly organized tissues. The vascular stasis that follows thawing constitutes a major mechanism of injury within the volume of previously frozen tissue thereby increasing the probability that cells die. While the relative importance of these two mechanisms of injury has long been debated, the two are clearly synergistic in cryoinjury leading to cell death from freezing [11–14].

Apoptosis or programmed cell death has been identified as a mechanism of cell death associated with thermal injury [15]. In investigations with human prostate cancer cells in vitro, Hollister et al. described cells dying from apoptosis some days after freezing [16, 17]. Experiments in vitro have shown that apoptosis occurs following exposure to modest freezing temperatures and that cells are susceptible to apoptotic initiation events up to 12–24 h after thawing [18–21]. Clarke et al. observed cell rupture and necrosis immediately post-thaw, while apoptotic cell death was prominent 12-h post-freeze [19]. Subsequent studies, apoptotic death showed this to be partially regulated through the mitochondria [21–23]. The mitochondria play an important role in the apoptotic death cascade, most notably through the influence of the Bcl-2 family of proteins, regulators of apoptosis [23–28]. More recently, Robilotto et al. identified a temporal wave of apoptosis induction initiating within an hour post-thaw at the core of the frozen mass when ultra-low temperatures are attained followed by the movement of apoptotic induction outward towards the periphery over the next 18–24 h [22]. Further, this study revealed that the rapid induction of apoptosis at ultra-low temperatures progressed through a membrane-mediated pathway whereas the delayed apoptosis in the periphery progressed through a mitochondrial-mediated pathway.

The Freeze-Thaw Cycle

Cryosurgical technique requires that tissue be rapidly frozen, thawed slowly and completely, and then exposed to a second freeze cycle so that the goal of achieving a temperature in the targeted tissue is attained along with a safe margin around the tumor [12, 14, 29]. Each of the multiple phases of the freeze-thaw cycle (i.e. cooling rate, tissue temperature, freezing duration, and thawing rate contribute to tissue injury) are highly damaging to cells. Repetition of the freeze-thaw cycle subjects the tissues to a repeat injurious paradigm important to complete tumor destruction. The characteristics of each of these phases of the cycle vary in relation to the distance from the cryosurgical probe. This cycle of freezing also allows for the driving of ablative isotherms (−20 °C or −40 °C) further out from the cryoprobe region helping increase the level of cell destruction [30].

Rate of Tissue Cooling

Rapid cooling increases the probability of lethal intracellular ice crystal formation. Intracellular ice formation typically occurs at cooling rates greater than 20 °C per minute [29, 31]. In clinical practice, the cryosurgical probe should always be used at the lowest attainable temperature to obtain a greater probability of intracellular ice formation since much of the frozen tissue volume will be subjected to only slow cooling rates. Additionally, studies have shown that the rapid induction of membrane mediated apoptosis at ultra-cold temperatures and rapid-cooling rates starts the cancer cell down an irreversible path to death therefore increasing the possibility of cancer destruction [22].

Target Tissue Temperature

Tissue temperature is the critical factor in the application of a cryosurgical technique. Cell death occurs in greater numbers as the tissue temperature is lowered toward a nadir. Cells from different tissue sources demonstrate different lethality ranges [8, 16, 32]. Those of dermatologic origin are typically the most sensitive to freezing while those of the prostate are far hardier. Most skin lesions are fully ablated at temperatures between −10 and −20 °C while certain prostate cancers require a range between −40 and −80 °C. Studies have also demonstrated that the molecular disposition of a specific cancer type can also influence the cells response to freezing. For instance, Klossner et al. demonstrated that early stage androgen responsive prostate cancer is more resistive to freezing injury than the late stage androgen non-responsive prostate cancer cells [32].

Duration of the Freeze-Thaw Cycle

Studies that would definitively establish the duration of freeze cycle (i.e. duration of nadir temperature holds during a single or double freeze and interval between first and second freeze cycle) are wanting. While longer durations are intuitively beneficial, only limited in vitro research [30, 33] provides quantitative data supporting physician practice. Hence, anecdotal evidence and physician instinct guide timing in regard to clinical practice. Hold times of a "few minutes" at the nadir temperature is thought to be adequate to assure that the targeted lesion is fully involved at the nadir and that local circulation is arrested. To this end, Klossner et al. demonstrated that hold times of 1–2 min at target temperature were adequate to result in cell death [30]. Holds of shorter duration resulted in less effective cell death where holds longer than 2 min at a given temperature did not increase the level of death. When a dual freeze-thaw cycle is applied, the thaw interval should be of adequate duration to assure passive thawing of the outer margin of the freeze zone. Passive thawing allows for prolonged exposure to the nadir temperature, which is elevated in comparison with the inner mass of the freeze zone. The repeat freeze cycle provides a double stress event to the cell population as well as allows for critical temperatures to be driven further from the cryoprobe thereby increasing the overall kill zone.

Thermocouple Monitoring

The use of thermocouples to monitor tissue temperature during freezing has emerged as an important adjunct to the imaging techniques. Needle-mounted thermocouples have proven accurate and useful for thermal monitoring, especially when inserted in critical areas [34]. Their use allows for the confirmation that lethal temperatures have been achieved in the target tissue or that injurious temperatures have not been reached in critical areas, such as in the wall of the rectum. It is important to note that thermocouples measure point sources of temperature within the tissue. As such, these temperatures cannot be extrapolated easily to the entire volume of the cryosurgical lesion.

Adjuncts to Cryoablation

Cytotoxic drugs when used as adjunctive agents offer a promising approach to increase the kill efficacy of cryotherapy along the margin of the ice ball [19–21, 23, 28]. Sub-toxic exposure to

agents such as 5-Flurouracil or Taxotere prior to the freezing insult can increase the lethal affect of freezing at the elevated sub-freezing temperatures found within the freeze zone periphery [19, 28]. The combined benefit of 5-FU and freezing is to increase the rate of apoptosis in the targeted tissue margin [19, 28, 35]. Other studies have shown that other agents such as Taxotere [23], cisplatin [16], vitamin D_3 [36, 37], TNF [38, 39], and TRAIL [40], among others, providing a synergistic benefit when used in conjunction with cryoablation raising the lethal temperature necessary from the −20 °C to −30 °C range to around −10 °C or warmer. These adjunctive strategies have shown promise to significantly improve tumor ablation.

Cryoablative Technologies

Beginning in the mid-1960s, cryoablation underwent a significant technical advancement [6] and now serves as an effective treatment modality for a number of cancers. Further technical modifications were realized in the 1990s including the development of new cryosurgical apparatus, imaging techniques, and adjunctive devices to improve the applicability and efficacy of cryotherapy. Technical improvements, such as the use of new multi-probe devices and the development and utilization of a protective urethral warming catheter may be cited as significant milestones in the evolution of cryosurgical technique [41–47]. Better selection of patients, with appropriate staging of disease, has substantially improved overall results.

Cryogen selection provides option to support diverse treatment of diverse clinical indication ranging from de-bulking to total ablation. Carbon dioxide, a cryogen with the most limiting ablative action (−78.5 °C), and nitrous oxide (−88.5 °C) find limited use. Argon (−185.8 °C) and liquid nitrogen (−195.8 °C) are more widely adopted in cryosurgical devices that operate with closed-end cryoprobes. LN is a conveniently managed liquid utilized in spray, wick and probe configurations. Recently, a next generation class of devices has been developed utilizing critical and supercritical cryogens, poised to provide far more rapid and effective freezing tissue thereby increasing the level of death while reducing the time and collateral damage associated with the freeze thaw process [48].

Modern Cryosurgery

The application of cryosurgery often relies on guidance from information derived from the imaging techniques. Ultrasound, allows for monitoring of ice ball growth progression, but has significant limitations because the practitioner cannot see beyond the nearest ice plane of frozen tissue. The resulting image is two-dimensional because acoustic shadowing precludes visualizations of the extent of freezing behind the ice front [45]. Three-dimensional ultrasound may well alleviate this problem [46, 47, 49, 50]. Another limitation of ultrasound occurs because the image provides no information about target tissue temperature, which causes difficulty in making real-time determination of where the critical −40 °C isotherm is within the ice ball. To address the issue of thermal monitoring, the use of thermocouples, in conjunction with ultrasound, has added an increased level of certainty of the success.

New directions in imaging for cryosurgery include computerized tomography (CT), magnetic resonance imaging (MRI), and electrical impedance tomography (EIT). CT has the benefit of showing the entire cross sectional image of the frozen tissue. The images are made at intervals of a minute or two, which is not real time but still within the realm of usefulness [51]. MRI provides a three dimensional view of the volume of frozen tissue and has shown promise as a clinically valuable monitoring technique in cryosurgery [52–55]. MRI data allow the temperature within the frozen volume to be established using mathematical models [56–59]. The techniques and tools for use with MRI are still evolving, as are the MRI contrast agents [60]. Harada et al. recently demonstrated the usefulness and safety of MRI-guided cryosurgery for renal tumors [61]. The probability of extensive or routine clinical use of MRI-guided cryosurgery in the near future is remote because of expense. Electrical

impedance tomography (EIT) has been proposed as a method of monitoring the freezing of tissue [62]. EIT provides a global image by introducing low amplitude AC currents into the body, thus measuring the electrical potentials on the body surface. These potentials are then recorded and analyzed to create a tomographic image [63–65]. This approach to imaging is new and needs further development prior to use in clinical-based cryosurgical procedures.

Summary

Tissue injury is produced by a sequence of destructive effects, beginning with prolonged tissue cooling, metabolic disruption, ice crystal formation and cellular rupture. After thawing, microcirculatory failure and the associated ischemia add to cell death, resulting in a coagulative necrosis. Physical processes of destruction are effective immediately, but physiological-based detrimental effects, including cytokine release and induction of apoptosis and secondary necrosis, produce a damaging effect over several days.

The basic principles of cryosurgery for tumors are fast cooling of the tissue to a cell-lethal temperature, slow thawing, and repetition of the freeze-thaw cycle. Ideally a temperature of −40 °C should be produced at the tumor margin to ensure that all portions of the tumor are subjected to lethal conditions. Repetition of freezing-thaw cycle elevates the cell-lethal temperature due to additive cellular stress, as does an increased duration of freezing.

In vitro and in vivo experiments on the molecular basis of cell death associated with cryosurgery have demonstrated the potential value of adjunctive cytotoxic chemotherapy. The idea is to increase the extent of injury to cells in the peripheral portion of the cryogenic lesion with the expectation that differences in cell sensitivity to freezing may be mitigated. The objective of these strategies is to "make ice lethal at 0 °C". This would provide for an ablative event throughout the entire target region, markedly reducing the potential of disease reoccurrence from satellite populations of cancer cells surviving within the targeted frozen region. Further research should lead to a better understanding of the molecular mechanisms involved in cryosurgery and adjunctive therapy, which in turn should increase the efficacy of cryosurgery for tumors.

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