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Cryoablation: Mechanism of Action and Devices

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Abstract

Cryoablation refers to all methods of destroying tissue by freezing. Cryoablation causes cellular damage, death, and necrosis of tissues by direct mechanisms, which cause cold-induced injury to cells, and indirect mechanisms, which cause changes to the cellular microenvironment and impair tissue viability. Cellular injury, both indirect and direct, can be influenced by four factors: cooling rate, target temperature, time at target temperature, and thawing rate. In this review, the authors describe the mechanisms of cellular injury that occur with cryoablation, the major advantages and disadvantages of cryoablation compared with other thermal ablation techniques, and the current commercially available cryoablation ablation systems.

CRYOABLATION refers to all methods of destroying tissue by freezing (1). Cryoablation has been applied to the treatment of cancer since the mid-19th century, when Dr. James Arnott used salt solutions containing crushed ice at -18° to -24°C to freeze breast, cervical, and skin cancers (2). He observed resultant shrinkage of the tumors and a significant decrease in pain. Although cryoablation has been used to treat malignancy in a wide variety of organs, including the eye, brain, head/neck, and esophagus, in current practice it is most commonly used in the treatment of liver, kidney, lung, prostate, and breast malignancy (3–8). Cryoablation can be performed via surgical (open or laparoscopic) or percutaneous approaches (9). Improvements in imaging, which allow earlier detection of smaller cancers, and a trend toward minimally invasive techniques in oncology, have made image-guided oncologic intervention like cryoablation an attractive alternative to surgical treatments.

MECHANISM OF ACTION

Thermodynamics

Percutaneous cryoablation is performed by inserting cryoprobes into malignant tissue under imaging guidance. After targeting the lesions with one or more cryoprobes, the cryoprobe is rapidly cooled, removing heat from the tissue by conduction via physical contact with the cryoprobe. Rapid cooling of the cryoprobe takes place by means of the Joule-Thompson effect, whereby rapid expansion of a gas that does no work (adiabatic expansion) results in a

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change in the temperature of the gas (10). Most gases, including oxygen, nitrogen, and argon, exhibit Joule-Thompson cooling when rapidly expanded at room temperature (eg, the cooling of an aerosol can when being sprayed). However, because of the unique physical characteristics of hydrogen and helium, these gases warm when rapidly expanded at room temperature. The cryoprobe is essentially a high-pressure, closed-loop, gas expansion system. When the high-pressure room temperature gas (typically argon) reaches the distal aspect of the cryoprobe, the argon is forced through a throttle (narrow opening) and then allowed to rapidly expand to atmospheric pressure. The rapid expansion of the argon causes a decrease in the temperature of the gas (the Joule-Thompson effect), which is rapidly transferred by convection and conduction to the metallic walls of the cryoprobe. The depressurized gas is vented back out of the hub of the needle. Warming of the cryoprobe and thawing of the tissue is performed through the same system using high-pressure helium, which warms the cryoprobe during expansion to atmospheric pressure.

Direct and Indirect Cellular Injury

Cryoablation causes cellular damage, death, and necrosis of tissues (Fig 1) by direct mechanisms, which cause cold-induced injury to cells, and indirect mechanisms, which cause changes to the cellular microenvironment and impair tissue viability (11). As the cryoprobe absorbs heat from the tissue, the tissue cools, eventually forming ice crystals in the extracellular space. The ice crystals sequester free water, which increases the tonicity of extracellular space. Osmotic tension draws free intracellular water from cells, dehydrating them (12). The concomitant increased intracellular solute concentration results in damage to cytoplasmic enzymes and the destabilization of the cell membrane. These effects may be mediated by the cold denaturation of proteins, whereby the 3-dimensional conformation of proteins is altered with cooling and dehydration (13). Because peptide bonds are not disrupted in the process, cold denaturation of proteins can be reversible with warming and rehydration.

When the cooling of tissues occurs rapidly, there is not enough time for intracellular dehydration. Free water is trapped within cells during the freezing process. In this setting, rapid cooling results in intracellular ice crystal formation, a harbinger of immediate cell death (14). Although the exact mechanism of cellular damage from intracellular ice formation is unknown, injury is thought to be mediated by physical damage to intracellular organelle membranes and the plasma membrane (15). Crystal-induced pore formation in the plasma membrane results in a loss of electro-chemical gradients, which prevents transport even after the cell has thawed. If the pores are large enough, cellular components can diffuse into the extracellular space. During thawing, melting ice within the extracellular space results in its hypotonicity with respect to the intracellular compartment; thus, an osmotic fluid shift can occur, leading to cell swelling or bursting. In addition, an influx of free water into the intracellular space can result in the growth of intracellular ice crystals, exacerbating their biocidal effects (16). The growth of intracellular ice crystals can occur even as melting proceeds in the extracellular space, as high extracellular solute concentrations lower the freezing point of water in the extracellular space. In fact, during thawing, intracellular ice crystal growth is maximized at -20° to -25°C (17).

Cells that are not immediately killed by direct cryoablation-induced injury may subsequently die by apoptosis, or programmed cell death (18). Despite preservation of the integrity of plasma membranes and continued cellular active transport, intracellular damage to mitochondria can signal activation of a family of cysteine-aspartate proteases (caspases). These caspase family proteins cleave various proteins, resulting in morphologic and biochemical changes of apoptosis characterized by cell shrinkage, membrane blebbing, chromatin condensation, and genomic fragmentation (19). Apoptotic cell death after cryoablation is seen typically in the periphery of ablation zones, where exposure to temperatures that are not immediately lethal results in irrecoverable cellular injury without immediate cell death (20).

Indirect cellular injury is the result of cold-induced changes to tissues causing an unfavorable microenvironment for cellular survival. Intracellular ice crystal formation in blood vessels causes damage to the vascular endothelial cells (21). In the post-thaw period, reperfusion brings in platelets, which contact the damaged endothelium, resulting in thrombus formation and resultant ischemia (22). The release of inflammatory cytokines begins a cascade of molecular events, which increases vascular permeability, producing tissue edema (16). Ischemia also results in the production of vasoactive substances, causing regional hyperemia. An influx of inflammatory cells, such as neutrophils and macrophages, ensues, which aids in the cleanup of cellular debris. This process can continue for weeks to months after the ablation, culminating with a zone of coagulation necrosis in the ablation zone, surrounded by a band of neutrophil in its periphery (22).

Cellular injury, both indirect and direct, can therefore be influenced by four factors: cooling rate, target temperature, time at target temperature, and thawing rate (16). The basis of the influence of each factor stems from the biology of cold-induced injury described above. A faster rate of cooling results in a higher intracellular water content before freezing, maximizing intracellular ice crystal formation (23). The rate of cooling will be most rapid adjacent to the cryoprobe and lower in the periphery of the iceball, where the greater surface area lowers the flux of heat removal. Concomitantly, the target temperature will be lowest adjacent to the cryoprobe and highest in the periphery of the iceball. Based on this fact, increasing the time at target temperature will increase the likelihood that cells in the peripheral portion of the ice ball will have a sufficient time and temperature for lethal intracellular ice to form (24). Another challenge to intracellular ice formation in the periphery of the cryolesion is a lowered intracellular freezing point caused by intracellular dehydration and increased solute concentration that occurs during slow peripheral cooling. In addition, cells from different organs, as well as different tumor types, display varied thresholds for cold-induced cell death (25–27). However, almost all tissues exhibit subsequent cell death when cooled rapidly below -40°C (28–29). Finally, the rate of thawing plays a role. Rapid thawing can increase the chance of cell survival by limiting the size of intracellular ice crystals. This idea is supported by studies that show a greater degree of cell death with passive thawing than with active thawing and that repeated freeze thaw cycles lead to a higher degree of liquefactive necrosis (27,30).

WHY CRYOABLATION?

Advantages

The primary advantage of cryoablation over other thermal ablation techniques is the ability to monitor the ablation zone during the procedure in real time (31). During freezing, the water of the tissue undergoes a phase transition from liquid to solid, forming an iceball, which is visible under ultrasound (US), computed tomography (CT), and magnetic resonance (MR) imaging guidance. Ice has a slightly lower density than water, due to a slight expansion during the phase transition from liquid to solid because of the crystalline structure of the ice. During CT, the ablation zone appears as a sharply demarcated hypoattenuating zone around the cryoprobe (32). The edge of the iceball marks the 0°C isotherm, where the phase transition occurs. By US, the superficial surface of the iceball appears as an hyperechoic rim, with intense acoustic shadowing that limits the ability to directly determine the depth of ice formation (33). MR monitoring of cryoablation can be performed with either T1- or T2-weighted sequences; the iceball appears as a dark signal void (34–35). Interestingly, because of the small amount of free water present in frozen tissue and the temperature sensitivity of its relaxation properties, MR thermography can be used to assess temperature gradients within the iceball (36).

Because the cooling of tissues and nerves provide an anesthetic effect, cryoablation tends to be less painful than the heat-based thermal ablation techniques like microwave or radiofrequency ablation. As such, cryoablation typically does not require general anesthesia and often can be performed in the outpatient setting with moderate sedation. Because the cooling mechanism is primarily mechanical rather than electronic, operation of cryoablation devices typically does not cause interference with CT or MR imaging machines, as is seen with radiofrequency ablation. In addition, each cryoablation probe acts independently of others, allowing multiple probes to be used simultaneously to create an ablation zone that conforms to the tumor being treated. Radiofrequency probes require a closed loop electric circuit, and although multiple probes can be placed in a single tumor, they must each be used sequentially rather than simultaneously. Given the novelty of cryoablation and the lack of randomized trials comparing cryoablation to other thermal ablation techniques, it has yet to be determined how cryoablation compares with other image-guided surgical therapies with regard to clinical efficacy.

However, a lower complication rate has been seen with cryoablation when compared with radiofrequency ablation in the treatment of renal cell cancer (37). One hypothesis to explain this finding is that freeze-induced cellular injury may be less destructive to some structural components of tissue than radiofrequency thermal ablation (38). This observation is supported by the porcine model of collecting system injury after both radiofrequency and cryoablation (39). However, collecting system injuries have been reported with renal cryoablation (40).

In contrast to radiofrequency ablation, cryoablation results in a robust inflammatory response (41). This may be the result of the maintained presence of tumor proteins that remain in situ after cryoablation compared with the coagulative type denaturing of proteins seen with radiofrequency ablation. Not surprisingly, tumor antigens in the presence of

inflammatory and immunomodulatory mediators create the potential to stimulate immunologic responses to tumor-specific antigens in ablated tissue, which has the potential to target and potentially kill tumor cells that are not in the vicinity of the ablation zone (42–43). In fact, cryoablation has been shown to produce antibodies to the ablated tumor antigen in both animals (44) and humans (45). In addition to potential humoral-mediated responses, animal studies suggest that a more tumoricidal cell-mediated immune response may be stimulated to a greater degree with cryoablation than radiofrequency ablation (46). A possible explanation is that the combination of the increased inflammation and the larger degree of in situ tumor antigen with cryoablation results in greater antigen presentation by dendritic cells, eliciting a more robust T-cell-mediated antitumoral response. Research directed toward adjuvant immunomodulatory therapies given concurrently or after cryoablation may prove beneficial in maximizing the efficacy of cryoablation (47).

Disadvantages

The inflammatory response after cryoablation can lead to a systemic inflammatory response syndrome termed *cryoshock* (48–49). This constellation of findings, which can include hypotension, respiratory compromise, multi-organ failure, and disseminated intra-vascular coagulation, is mediated by cytokine production (50). It is seen typically with large-volume liver cryoablation. Because cryoablation does not use heat, cautery effects and coagulation of injured vessels do not occur, which can exacerbate bleeding complications. Frozen tissues are more brittle than heated tissues, and excessive torque or displacement of cryoprobes while in the tissue can result in organ fracture (51), which can lead to significant bleeding. Aside from the ablation needle and machinery required of all ablation modalities, cryoablation also requires purchase and storage of sufficient quantities of argon and helium gas, which can cost up to \$200 per case.

CRYOABLATION SYSTEMS

Currently, 2 cryoablation devices are available commercially in the United States, both of which cool tissue using the Joule-Thompson effect. Healthtronics, formerly Endocare, produces the Percryo system (Healthtronics, Austin, Texas), which allows placement of up to eight individually controlled cryoprobes. Needle sizes range from 17 to 24 mm in diameter. The size and shape of the resultant iceballs are a function of cryoprobe size and Joule-Thompson chamber configuration. Galil Medical's cryoablation system (Galil Medical, Arden Hills, Minnesota) relies on 14.7-mm probes that have the added advantage of being MR compatible. This system also enables real-time monitoring of temperature using available thermocouples.

New cryoablation devices under development seek to overcome some of the difficulties that arise when cooling tissue using the Joule-Thompson effect. Rare noble gases like argon and helium are expensive, and attempts have been made to create systems that cool needles by simply circulating a cold liquid cryogen. The challenge of this type of system is that as the cryogen chills tissue at the tip of the cryoprobe, absorption of heat from the tissue causes a phase transition in the cryogen from liquid to gas. This rapid expansion of gas at the tip of the cryoprobe can block the flow of the liquid cryogen through the needle, an effect termed

vapor lock. However, by circulating pressurized nitrogen near its critical point, which refers to the point at which there is no distinct phase transition between liquid and gas, vapor lock can be avoided. In this way, large amounts of expanding gases are not lost, as is seen with devices that utilize the Joule-Thompson effect, creating the potential for significant cost and space savings.

CONCLUSION

The biology that underlies the cold-induced injury to tumors directly affects the parameters by which image-guided cryoablation is performed: cooling rate, target temperature, time at target temperature, and thawing rate. Future adjuvant therapies to cryoablation will rely on understanding these relationships to maximize procedural efficacy and minimize procedural morbidity. Although it is certain that the technologies to perform cryoablation have great potential for evolution, cryoablation of malignancy has already become established as an important modality in image-guided interventional oncology.

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Figure.

Cryoablation-induced injury. **(a)** During freezing, extracellular ice formation results in sequestration of free extracellular water, increasing the osmolarity of the extracellular space. This leads to cellular dehydration and cell shrinkage. Intracellular ice formation results in disruption of organelle and plasma membranes, impairing cellular function. **(b)** During thawing, extracellular ice melts before intracellular ice, creating an osmotic fluid shift of water into damaged cells, causing swelling and bursting. Growth of intracellular ice crystals can continue during thawing, exacerbating cellular damage. **(c)** Damage to the vascular endothelium results in tissue edema. Delayed cellular damage occurs because of the initiation of apoptosis by the cold-induced cellular injury. Thrombosis of blood vessels causes tissue ischemia, hindering repair. Inflammatory cells, including macrophages and neutrophils, remove damaged cells and clear cellular debris.