Radiofrequency ablation of liver tumors (I): biological background

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Summary. *Majority of patients suffering from liver tumors are not candidates for surgery. Currently, minimal invasive techniques have become available for local destruction of hepatic tumors. Radiofrequency ablation is based on biological response to tissue hyperthermia. The aim of this article is to review available biological data on tissue destruction mechanisms. Experimental evidence shows that tissue injury following thermal ablation occurs in two distinct phases. The initial phase is direct injury, which is determined by energy applied, tumor biology, and tumor microenvironment. The temperature varies along the ablation zone and this is reflected by different morphological changes in affected tissues. The local hyperthermia alters metabolism, exacerbates tissue hypoxia, and increases thermosensitivity. The second phase – indirect injury – is observed after the cessation of heat stimulus. This phase represents a balance of several promoting and inhibiting mechanisms, such as induction of apoptosis, heat shock proteins, Kupffer cell activation, stimulation of the immune response, release of cytokines, and ischemia-reperfusion injury. A deeper understanding of the underlying mechanisms may possibly lead to refinements in radiofrequency ablation technology, resulting in advanced local tumor control and prolonged overall survival.*

Hepatocellular carcinoma (HCC) is one of the most common solid cancers, with an annual incidence estimated to be at least one million new patients, especially in Eastern Asian and African countries (1). Furthermore, the liver is second only to lymph nodes as a common site of metastasis from other solid cancers, particularly in patients with colorectal carcinoma (CRC) (2). Surgical resection of HCC and hepatic metastases from primary tumors with liver-only metastases can result in significant long-term survival benefit in 20% to 35% of patients (3). However, a substantial number of patients are not candidates for surgery. Only 5% to 15% of patients with newly diagnosed HCC or CRC liver metastases undergo potentially curative resection. Difficulties related to surgical resection are those of the size, number of tumors, location (adjacent to porta hepatis), extrahepatic involvement, poor general condition, and poor liver function especially in HCC with inadequate functional hepatic reserve related to coexistent cirrhosis. Currently, minimal invasive techniques have become available for local destruction of hepatic tumors. These are percutaneous ethanol injection (PEI), hepatic arterial chemoembolization, and local thermodestruction.

Focal hyperthermia may be induced by several methods and is at present the preferred local ablative technique. These methods (radiofrequency, microwave, interstitial laser thermotherapy, high-intensity focused ultrasound) vary according to the processes of heat generation and its delivery. The energy delivered to the liver tumors in minimally invasive fashion causes tumor destruction with no significant damage to normal liver parenchyma. The aim of this article is to review the general principles of local heat production and to systemically present available biological data on tissue destruction mechanisms.

Radiofrequency ablation (RFA) has emerged as the most widely accepted method for both palliative and curative strategies. RFA uses electromagnetic energy sources to generate heat. Electrode probes are placed within tumors percutaneously or during open or laparoscopic surgery. High-frequency (375–500 kHz) electromagnetic waves displace molecules within the tissue in alternating directions. Changes in the energy state of molecules result in an ionic agitation and localized increase in temperature, reaching approximately 90˚C (4).

In comparison with other minimally invasive methods, such as PEI or transarterial chemoembolization,

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RFA has been shown to achieve similar results in fewer therapeutic sessions. Moreover, RFA seems to be superior to PEI in terms of long-term survival and local recurrence rate (5). On the other hand, PEI seems to be superior to RFA in a subcapsular tumor location, when tumor is adjacent to a major vessel or another organ (heart, gallbladder, stomach, and bowel), and when demonstrating poor differentiation (6). The retrospective nonrandomized studies revealed that RFA has lower local recurrence and complication rate when compared to cryotherapy (7). The main advantages of RFA include low morbidity and mortality rates, effective tumor ablation, as well as the most efficient preservation of normal liver parenchyma. This is particularly important in patients with limited liver function reserve.

Although the principles of heat production are different, the mechanisms of thermal tissue destruction or damage are similar. It has been shown that tissue injury following thermal ablation occurs in two distinct phases. The initial phase is *direct injury*, which is predominately determined by the total energy applied, tumor biology, and tumor microenvironment. The second phase is *indirect or progressive injury*, which follows the cessation of the focal hyperthermic stimulus.

The direct thermal injury involves all the reactions in tissue at cellular and subcellular levels. The tumor biology and microenvironment are very important in this process. The tissue effects depend on energy applied, the duration of thermal injury, thermal sensitivity of cells, and characteristics of heat removal mechanisms, which vary in different organs and tissues (8).

The keystone mechanism of tissue injury involves the inactivation of vital enzymes. The temperatures between 100˚C and 300˚C induce vaporization of tissue water, carbonization, and smoke generation (9). The carbonization limits the extent of tissue damage by creating a heat trap. The carbonization process may result in tumor cell dissemination as a result of increased interstitial pressure. In clinical setting, the heat transfer is improved by tissue infiltration with saline, which prevents tissue carbonization and safeguards against excessive increase in impedance. Near immediate coagulation of tissue is induced at temperatures between 60˚C and 100˚C and manifests as an irreversible damage to mitochondrial and cytosolic enzymes of the cells. The temperature decreases away from the active probe and is reflected by different morphological changes in affected tissues (10). These changes are morphologically described as four zones: application, central, transition, and reference tissue zones. *The application zone* is located immediately to the thermal electrode and is characterized by the irreversible damage (carbonization). The necrotic tissue with damaged structure of hepatic cells is observed in *the central zone. The transition zone,* surrounding the central zone, is morphologically described as hemorrhagic area, containing apparently undamaged liver cells with signs of tissue infiltration with blood cells. In this zone, cells are eosinophilic with condensed chromatin; however, intercellular connections are maintained. *The reference zone* referrers to the normal tissue surrounding the transition zone (11).

The most complicated processes of thermal tissue damage are observed in the transition zone. This zone contains cells in the earliest stages of cellular death, demonstrating loss of enzymatic activity. The direct effect of heat stimulus at the subcellular level results in impaired membrane and mitochondrial function, destruction of nucleic acids and cytoskeleton. Cell viability accurately correlates with ultrastructural changes in the mitochondria. Formation of intramitochondrial dense granules, vesicularization of the crista, intracristal space swelling, and myelin degeneration are observed. These changes promote proton leakage across the inner mitochondrial membrane and impairs oxidative phosphorylation (12). Histological techniques are not appropriate to assess cell viability immediately after heat stimulus. While major ultrastructural changes in mitochondria are observed within 15 min of heat injury, mitochondrial viability may be assessed histochemically from this time point. This involves staining for mitochondrial enzymes, including nicotinamide adenine dinucleotide diaphorase (NADH-diaphorase), cytochrome c oxidase, succinic dehydrogenase, lactate dehydrogenase. Staining for NADH-diaphorase is the most popular test. Activity of this particular enzyme ceases immediately following irreversible cellular injury. Staining for NADH-diaphorase activity clearly discriminates viable from nonviable tissue before morphological changes of necrosis become apparent (13).

The thermosensivity of the tumor is a type-specific feature independent of the proliferation rate, absorbance, and nature (benign or malignant) of the cells. It may reflect differences in the structure and size of cellular components including the distribution of intermediate filaments that alter susceptibility to heat injury (14). Tumors have a higher fraction of cells in the mitotic or S-phase of replication, lower mitochondrial levels, reduced intracellular adenosine triphosphate (ATP), and lower overall oxygen intake compared to normal cells. Hyperthermia enhances

oxygen consumption, resulting in higher susceptibility of these cells to hyperthermia.

Enhanced tissue destruction in this zone is facilitated by peculiarities of tumor vasculature. It has been shown that tumors have a lower blood flow when compared with normal liver tissue (15). Moreover, tumor vessels are usually close to maximal dilation; consequently, they are more sensitive to thermal injury when compared to that in normal liver and more likely to undergo irreversible injury. An irreversible decrease in tumor blood flow with vascular stasis and thrombosis has been described at the temperatures between 42˚C and 44˚C. The extent of vascular occlusion is equivalent to the tissue injury of the tumor; however, the extent of vascular damage is significantly less than the tissue necrosis in normal liver parenchyma (16).

The relationship of major vessels to the extent of tumor necrosis in the liver is well established. Thermal injury is generally least efficient in tumors adjacent to large intrahepatic vessels due to the "heat sink" (17). Heat is absorbed by circulating blood and is transferred away, thus limiting local increase of temperature as well as the depth and extent of morphological changes of tumor tissue.

Application of local hyperthermia alters metabolism, exacerbates tissue hypoxia, decreases pH, and increases thermosensitivity, possibly through processes of direct enhancement of thermal killing, impairment of DNA synthesis, and reduction of cellular proliferation (18).

Indirect, *or progressive injury*, is observed after the cessation of direct heat stimulus. The full extent of tissue damage becomes evident in 24–168 hours (19). This injury may be a major determinant of completeness of tumor ablation. The lesion size decreases slightly during the course of several weeks as a result of the breakdown and removal of necrotic tissue by inflammatory cells.

The progression of injury appears to be independent of the initial thermal effect and is defined as ongoing necrosis. It is characterized by homogenous cytoplasmic eosinophilia, nuclear staining, and blurred cytoplasm borders. However, morphological distinction between complete necrosis and ongoing necrosis is sometimes difficult, necessitating histochemical demonstration of mitochondrial enzymatic activity. The ongoing necrosis is revealed using antibodies detecting single-stranded (denaturated) DNA (ssDNA) and the anti-human mitochondria antibodies. Itoh et al. used a polyclonal rabbit anti-ssDNA to label the nuclei of cells in ongoing necrosis, and mouse anti-human mitochondria monoclonal antibody MAB 1273 and anti-mitochondrial antibody 113-1 to stain viable cells (20).

A true injury progression represents a balance of several promoting and inhibiting mechanisms, such as induction of apoptosis, heat shock proteins (HSPs), Kupffer cell activation, stimulation of the immune response, release of cytokines, and ischemia-reperfusion injury.

Apoptosis is a distinctive form of cell death manifested by characteristic chromatin condensation and DNA fragmentation. Hyperthermia, changes in tissue microenvironment, release of various cytokines may trigger apoptosis. The induction of apoptosis at a distance from the site of heat application may potentially contribute to the progression of injury. The peak of apoptosis is observed in 2 h following local heat application (21).

Intracellular levels of HSPs are known to be important in the regulation of tumor cell thermosensivity (14). HSPs are produced as a result of stress. The baseline level of HSP70 and its expression following heat exposure vary with different cell lines and may influence the extent of protein loss and degradation (22). The expression of HSP 70 prevents cytochrome c/dATP-mediated caspase activation in vitro and inhibits apoptosis. The peak levels of HSP70 are observed in 10 h following heat stimulus (23). On the other hand, HSPs are shown to be involved in tumor antigen presentation and immune response modulation. HSPs and any abnormal peptide complex are transported across to the surface of the cell and binds to the CD91 receptor on the antigen presenting cell (APC) surface. This way T-lymphocyte and macrophage activity is stimulated, followed by increased immunoblast and mast cell levels by 20–28 days after the heat application, and this effect seems to be maintained up to 8 weeks (24). Kupffer cells are also involved in progressive tissue injury via active phagocytosis of cancer cells in vivo and impeding formation of metastases (25). However, hyperthermia reduces activity of Kupffer cells, which is known to be important in the regulation of tumor growth by producing tumoricidal cytokines, such as IL-1, TNFα, and interferon. IL-1 and TNFα are involved in apoptosis. Increased levels of TNFα exhibit direct cytotoxic effects inducing endothelial injury, thus sensitizing tumor cells to hyperthermia (18). Interferon increases the liver-associated natural killer cell activity (26). Focal hyperthermia has been shown to modulate the expression of some growth factors. The expression of fibroblast growth factor (FGF) may cause the proliferation of residual tumor cells and have cytoprotective effects (27). Epidermal and hepatocyte growth factors

favorably influence liver regeneration and potential tumor growth (28).

The initial injury of microvasculature is followed by a progressive injury that peaks by 48 hours after treatment. The decrease in blood flow continues for 4–6 hours following heat application due to endothelial injury, increased vessel permeability, and blood viscosity, subsequently resulting in thrombus formation and ischemia. However, the progressive microvascular and tissue injury is more extensive in normal liver tissue as compared to liver tumors (16).

Concluding remarks

The success of local thermal ablation depends on ability to create adequate volumes of tissue destruction. Moreover, an additional area of apparently normal tissue adjacent to the tumor ("safety margin") should be ablated to eliminate microscopic foci of disease. Unfortunately, the exact underlying mechanisms of tumor destruction by focal hyperthermia and its influence on tumor growth are not precisely known. Despite continuing investigations of the biological processes induced by hyperthermia, the clinical results of radiofrequency ablation appear to remain unsatisfying in terms of tumor recurrence. A deeper understanding of those underlying mechanisms may possibly lead to refinements in radiofrequency ablation technology and energy delivery, resulting in advanced local tumor control and prolongation of an overall survival.

Kepenų navikų radiodažninė abliacija (I): biologinis pagrindimas

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Raktažodžiai: radiodažninė abliacija, kepenų navikas, terminis pažeidimas.

Santrauka. Tik nedaugeliui pacientų, sergančių kepenų navikais, galimas radikalus chirurginis gydymas. Daliai neoperuotinų ligonių taikomi lokalios navikų destrukcijos metodai. Radiodažninė abliacija pagrįsta biologiniu atsaku į audinių hipertermiją. Šio straipsnio tikslas – apžvelgti audinių termodestrukcijos biologinius mechanizmus. Terminis audinio pažeidimas vyksta dviem frazėmis. Tiesioginio pažeidimo fazę sąlygoja perduotos energijos kiekis, naviko biologija, jo mikroaplinkos ypatybės. Abliacijos zonoje temperatūra nevienoda ir tai sąlygoja skirtingus morfologinius pažeidimo zonos pokyčius. Lokali hipertermija pakeičia metabolizmą, pagilina audinio hipoksiją, padidina jautrumą temperatūrai. Antroji, netiesioginio pažeidimo fazė, prasideda pasibaigus šilumos poveikiui. Šios fazės metu įsijungia keletas reguliuojančių mechanizmų, tokių kaip apoptozės indukcija, šilumos šoko baltymų ir Kupfferio ląstelių aktyvacija, imuninio atsako stimuliacija, citokinų išsiskyrimas, išemijos-reperfuzijos pažeidimas. Gilesnis atsako į hipertermiją mechanizmų supratimas turėtų sąlygoti radiodažninės abliacijos technologijų tobulinimą, visavertiškesnę lokalią naviko destrukciją ir ilgesnį išgyvenamumą.

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