Solid Organ Cool-tip Radiofrequency Ablation: An Experimental Study with Clinicopathological Correlations

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Abstract. *Background/Aim: Radiofrequency ablation (RFA) with internally cooled electrodes is a technique for the in situ treatment of solid tumors, inducing characteristic pathological changes with limited clinical complications. Our purpose was to assess RFA-induced histological alterations and correlate them with clinical complications. Materials and Methods: Using a porcine model, the pathology of RFA-induced kidney, liver and spleen lesions was associated with the postoperative course and clinical complications recorded. Results: Complications and relevant histological lesions, including abscess formation, hemorrhage and bile or urinary leakage, were limited or absent. The majority of RFA-induced necrotic tissue exhibited preserved architecture, with relatively limited inflammatory reaction, associated with sealing of blood/bile vessels or urinary tubules along the periphery of the lesions. Conclusion: The preserved architecture of RFA-induced necrotic tissue, its slow clearance, the relatively limited inflammation and the ability of RFA to seal blood/bile/urinary vessels are probably responsible for the minimal complications observed.*

Radiofrequency ablation (RFA) is a method for the treatment of neoplastic lesions, including renal and liver tumors. Its effects are accomplished by increasing the tissue temperature and inducing protein denaturation and coagulative necrosis. The use of electrodes with an internally-cooled tip, capable of distributing the heat deeper in the tissue, results in ablation of larger volumes (1, 2). Apart from tumor ablation, RFA has been used for the treatment of hemobilia secondary to hepatocellular cancer (3), for controlling post-biopsy bleeding (4) and for reducing blood loss during segmental liver resection (5). Moreover, RFA has been applied in spleen surgery for the treatment of splenic trauma or hypersplenism in experimental models (6, 7).

Although RFA is an ablative technique causing thermal tissue damage, necrosis and inflammatory reaction, it does not usually induce a clinically detectable inflammatory process, abscess formation, hemorrhage, bile or urinary leakage (8-10).

In order to examine this discrepancy, we used a porcine RFA model, recorded the post-RFA clinical course, the complications and the post-mortem laparotomy findings of treated animals, investigated the histopathology of the lesions in detail and correlated the histological findings with the post-treatment clinical observations.

Materials and Methods

Animals and induction of RFA. Eighteen Landrace white male domestic pigs (average weight: 18 kg), randomly allocated into three groups of six animals each, were used (source: official provider of Prefecture of East Attica, European Ref. Number EL 090011). The first group (group L) underwent RFA in the liver, the second (group K) in the kidney and the third (group S) in the spleen. Three animals in each group were sacrificed on the third postoperative day (groups L1, K1 and S1) and the remaining three (groups L2, K2 and S2) on the 40th. All animals were sacrificed by administering 1 g thiopental *i.v.* under general anesthesia.

A Radionics Cooltip RF System (RADIONICS, USA; TYCO Healthcare, Athens, Greece) with a single shaft 15-cm needle-electrode with 2 cm exposure tip was used and "Impedance control" mode was selected. The ablation of the selected tissue lasted 12 min, similar to the time necessary for the ablation of metastatic lesions in humans, as previously described (6).

In order to carry-out RFA in the liver, the electrode was inserted through the superior surface of the center of segment V and advanced to the center of the selected area. For RFA in the right kidney, the electrode was placed in the center of the superior pole through the anterior surface, invading the collecting system. For the spleen, the lower pole of the organ was identified and the electrode was inserted into the center of it. All abdominal incisions were closed and the animals remained under close clinical observation, until sacrifice.

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Table I. *Comparison of radiofrequency ablation-induced pathological changes in all three organs on day 40.*

	Zone		
	1	2 (Same in all organs)	3
Liver	Preserved tissue architecture, cytoplasmic enlargement, eosinophilia, clotted vessels at the periphery	Zone 2a (closest to zone 1): Band of neutrophils digesting necrotic tissue and migrating towards zone 1	Reversibly damaged tissue (non-specific reactive hepatitis), bile extravasation and phagocytic reaction
Kidney	Preserved architecture, cytoplasmic enlargement, eosinophilia, clotted vessels at the periphery, lesion not sparing collecting system	Zone 2b: Loose hemorrhagic–inflammatory connective tissue Zone 2c (closer to the periphery): Dense fibrotic–inflammatory tissue with multinucleated giant cells disintegrating parenchymal remnants	Reversibly damaged tissue, interstitial nephritis
Spleen	White pulp: Preserved architecture, cytoplasmic enlargement, eosinophilia, clotted vessels at the periphery	Red pulp: Fully homogenized areas	Congested parenchyma only, small in size

All animal procedures were performed under general anesthesia. The protocol was approved by the General Directorate of Veterinary Services (licence No. 405), according to Greek legislation regarding ethical and experimental procedures (Presidential Decree 160/1991, in compliance with the EEC Directive 86/609 and Law 2015/1992 and in conformance with the European Convention "for the protection of vertebrate animals used for experimental or other scientific purposes, 123/1986". Animal handling and care was in accordance with National and European legislation about experiments in animals. This study was performed at the Experimental Research Center of ELPEN, which is located in the region of Attica. (European Ref Number EL 09 BIO 03).

Autopsy and histological examination. Autopsy and pathological studies were performed as previously described (6). Briefly, the target organ, the area of the operation and the whole peritoneal and thoracic cavities were thoroughly investigated. The respective organs were harvested, cut appropriately and fixed in 10% buffered formalin solution. Tissues were thoroughly sampled (at least one paraffin block per centimeter of damaged tissue), routinely processed and embedded in paraffin. Sections 4 μm-thick were stained with hematoxylin-eosin, periodic acid-Schiff (PAS), Gomori's reticulin and Masson's trichrome stains and evaluated by light microscopy. Evaluation of eosin and hematoxylin (H&E)-stained sections provided information about the intensity and extent of inflammation, the amount of necrotic tissue and the histological alteration of the viable tissue. The amount of fibrosis was evaluated according to Masson's trichrome and Gomori's reticulin stain. PAS stain, in addition to Gomori's and H&E stains, assisted in the evaluation of the integrity of epithelium basal membranes and sclerosis of kidney glomeruli. Evaluation was qualitative, or semiquantitative (mild, or intense inflammation and fibrosis) as provided in the Results section.

Results

Postoperative clinical course, postmortem laparotomy and macroscopic findings. Postoperatively, all animals appeared clinically healthy and in good condition. No loss of weight,

gross hematuria or other abnormal signs and symptoms were observed.

At postmortem laparotomy, no blood, bile, urine, pus or other fluids were identified. The small intestine was invariably adherent to the ablated area of the target organ (liver, kidney or spleen). After careful incision of adhesions, the ablated surface of the organ was freed and thoroughly inspected. No subcapsular or perisplenic hematomas were found in group S animals. One biloma (1.5 cm in diameter) was encountered in an animal of group L2. Finally, in one animal of group K2, a fistulous lesion leading to the collecting system was identified but no urine leakage was observed, due to obstruction of its orifice by the adherent small intestine.

Macroscopically, on day 3 after RFA, the ablated area appeared slightly paler than the normal tissue but the margins between necrotic and viable tissue were indistinct. At 40 days, the ablated area appeared solid and considerably paler in color than normal parenchyma. It was sharply demarcated by a yellow-whitish band, 1-2 mm in width and always marked by an elevation of the organ capsule.

Histopathology. Microscopically, a pale zone consisting of parenchymal cells with cytoplasmic enlargement and eosinophilia, indicating necrotic changes, was observed around the site of insertion of the electrode in groups L1, S1 and K1, on the third postoperative day. Tissue architecture was preserved. At the periphery, there was a band of congested blood with signs of an evolving inflammatory process, separating the inner necrotic zone from the outer normal-appearing tissue.

On day 40 (groups L2, S2 and K2), it was possible to identify three distinct areas (referred hereafter as zones) (overview in Figure 1 and comparatively described in Table
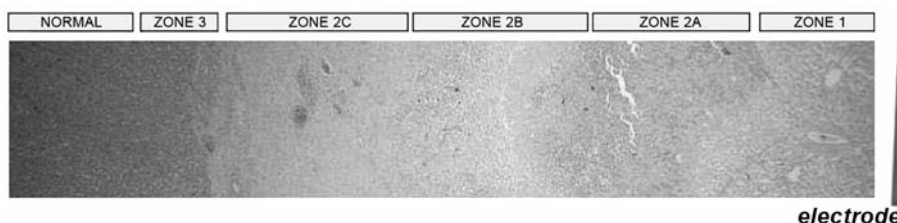
Figure 1. *Overview of histological alterations of liver tissue 40 days after radiofrequency ablation. Zone 1: Necrotic ghost-like tissue with preserved architecture. Zone 2a: Mainly consisting of neutrophils migrating towards zone 1. Zone 2b: Loose hemorrhagic–inflammatory connective tissue. Zone 2c: Dense fibrotic inflammatory tissue. Zone 3: Reversibly damaged tissue. Hematoxylin and eosin stain; original magnification ×40.*
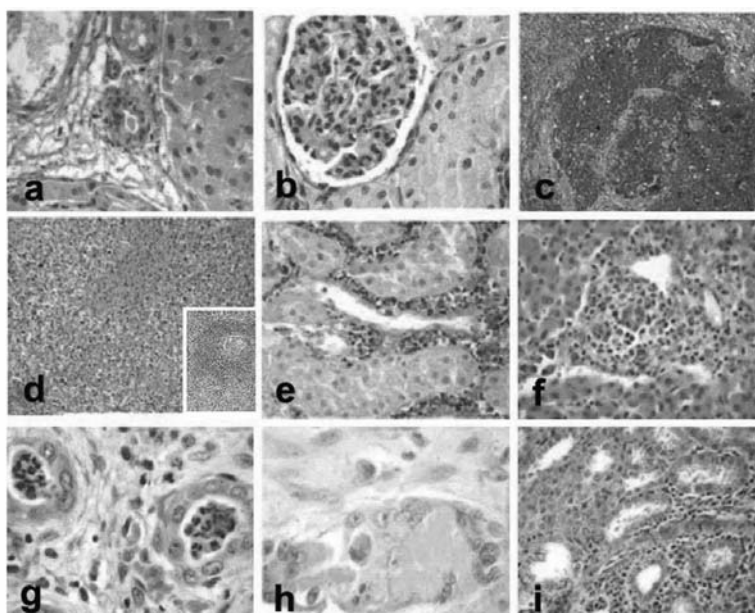

Figure 2. *Detailed histological alterations at day 40 after radiofrequency ablation. a: Necrotic liver parenchyma in zone 1 with ghost-like appearance. b: Necrotic renal parenchyma in zone 1 with ghost-like appearance. c: Thrombosed renal blood vessel next to necrotic renal parenchyma (Mason's trichrome stain). d: Homogenized splenic parenchyma in zone 1; inset: focal architectural preservation of white pulp inside the necrotic zone 1. e: Neutrophils at the front of zone 2 migrating towards the renal necrotic zone 1 (zone 2a). f: Limited non-specific hepatitis in liver zone 3. g: Focus of cholangiitis at the periphery of liver zone 2. h: Bile extravasating at the vicinity of cholangitis. i: Limited interstitial nephritis in renal zone 3. Eosin & hematoxylin stain; original magnification: a, b, e, f, g, h, i: ×400 and c, d, d-inset: ×100).*

I): Zone 1 of the liver (group L2) corresponded to the area of ablated tissue. It exhibited preserved architecture and the same cellular characteristics, as described above for the third postoperative day (Figure 2a). Kidney lesions demonstrated the same features and necrosis extended to the corresponding medullary area and renal papilla (group K2) (Figure 2b). Clotted blood vessels were observed at the periphery (Figure 2c). Zone 1 of the spleen (group S2) was also necrotic with preserved architecture, especially in white pulp, with nearly homogenized areas of red pulp exhibiting more typical features of coagulative necrosis (Figure 2d).

Zone 2 had the same characteristics in all three organs and was subdivided in three bands (a to c). Zone 2a (the one closest to the center) consisted mainly of a band of neutrophils migrating towards zone 1 and digesting the necrotic tissue (Figure 2e). Zone 2b consisted of looser hemorrhagic inflammatory connective tissue. Zone 2c, the thickest, consisted of dense fibrotic-inflammatory tissue containing parenchymal remnants (Figure 1). Multinucleated giant cells disintegrating calcified parenchymal cells were observed in all cases.

Zone 3 in the liver (group L2) corresponded to an area of reversibly damaged tissue appearing as non-specific reactive

hepatitis (mild chronic inflammation in portal tracts without fibrosis) (Figure 2f). However, localized bile extravasation at the margins towards zone 2 accompanied by phagocytic reaction was also seen (Figure 2g and h). Similarly, interstitial nephritis was seen in zone 3 of the kidney (group K2) (Figure 2i). With regard to the spleen (group S2), this zone was trivial, corresponding to minimally congested splenic parenchyma. The tissue in the periphery of zone 3 in all three organs corresponded to normal appearing, non-ablated parenchymal tissue.

Discussion

It is rational to hypothesize that tissue ablation induces an inflammatory and reparative process due to cell death, culminating in reabsorption of the dead tissue. An intense inflammatory process due to necrosis of a large amount of tissue could potentially result in rapid resorption of the ablated tissue, dead-space creation and facilitation of non-infectious abscess formation. A situation like this does occur as often as expected with RFA. We were not able to find any abscess formation case clinically, at postmortem laparotomy, nor at microscopic examination. Our results are in accordance with the findings of other investigators reporting complications in humans: De Baere *et al.* found 2% probability of abscess formation in their series of 350 sessions of RFA for liver tumors (11). Jansen *et al.* reported two abscesses at RFA sites in 143 liver RFA procedures (12). Wood *et al.* reported a less than 3.3% rate of abscesses (13) and other investigators reported an incidence of liver abscess of less than 1% (14).

The microscopic examination, almost one and a half month after RFA, revealed that the largest part of the necrotic ablated tissue had not disintegrated. Instead, it exhibited preserved architecture with a ghost-like appearance and the inflammation was limited to the periphery of the lesion, with inflammatory cells migrating gradually towards the center of the lesion and slowly disintegrating the dead tissue. It is possible that protein denaturation and enzyme inactivation induced by heat produced by RFA resulted in this fixed-like appearance of the dead tissue with minimal disintegration and the induction of a more controlled inflammatory process migrating from the periphery towards the center, without abscess formation.

No animal in our experiment suffered a hemorrhagic event and thrombosed vessels were invariably observed microscopically in the periphery of the ablated area in all studied organs. Hemostatic effect of RFA may originate from protein denaturation and coagulation necrosis of the vessel wall and vessel contents without vessel rupture and clot formation. RFA energy seems to be capable of sealing blood vessels; however, whether the activation of the clotting cascade is absolutely necessary is still unclear. It is interesting

to note though that at our Institution, patients with cirrhosis (known for their hemorrhagic diathesis) undergoing RFA-assisted liver resection do not seem to carry a significantly increased risk of bleeding, although this issue merits further investigation. Bleeding is a fairly well-investigated complication in the literature. Intra-abdominal hemorrhage was reported at 1.6% rate in a patient series after RFA treatment of liver lesions (15). Moreover, RFA through laparotomy was effectively performed for hemostasis on ruptured hepatocellular carcinoma, on an emergency basis, in a report by Ng *et al.* (16). Small perinephric hematomas were seen on computed tomographic scans and were confirmed at autopsy but without major hemorrhage in an animal model after kidney RFA in a study by Crowley *et al.* (17). Moreover, our team was able to control bleeding of traumatic etiology in porcine experimental models (10, 18, 19). This modality has also been used experimentally for sutureless laparoscopic partial splenectomy or liver segmentectomy and in RFA-assisted liver resection of all types in our clinical practice (20 and unpublished experience).

Bile leakage and biloma formation is another potential complication of RFA. Bile is incapable of forming clots and the prevention of bile leakage depends solely on the preservation of duct wall integrity, the denaturation of bile duct walls and the inflammatory fibroblastic reaction. Coagulative necrosis did not induce rupture of bile ducts in our study. Signs of chronic inflammation and fibrosis were evident in the periphery of the ablated area after a period of time (zone 2). We were able to find only one animal with biloma formation, a fact that indicates that if some bile leakage is present, it is quickly contained by inflammatory and fibrous tissue. The bile extravasation seen in zone 3 of animals which underwent liver RFA was of a minimal degree and had no clinical importance. Our findings are in accordance with those reported in the literature. Bile peritonitis occurred rarely in Tateishi *et al.*'s series (8). Similarly, one bile duct injury and subsequent abscess formation in one patient was reported among 91 RFA sessions by Wood *et al.* (13). Machi *et al.* (1 bile leakage in 60 operations for RFA) (9), De Baere *et al.* (1 bile leakage in 350 sessions) (11) and Bleicher *et al.* (fewer than five cases out of 198 procedures) (21) reported similar findings.

Urine leakage and urinoma formation comprise other significant complications that may increase morbidity after RFA procedures on the kidney. Although invasion of the collecting system by the tumor or the ablative radiofrequency energy is frequent, calyces and renal pelvis tend to seal. Janzen *et al.* suggested that RFA thermal injury does not spare the collecting system but healing or regrowth of the urothelium may occur with time after RFA (22). It is probable that the sealing of urinary tracts or the collecting system relies on inflammation and coagulative necrosis of the surrounding tissue only, since urine is not capable of clot formation. We

encountered only one urinary fistula among the animals of group K and other investigators were hardly able to find urinary leakage complications in their clinical or animal studies (10, 17).

In conclusion, RFA appears to be an effective and safe alternative for the treatment of neoplastic lesions of the liver and kidney. Regarding the spleen, although RFA has only rarely been used, its application seems promising. The sealing effects of tissue coagulation due to the increase of temperature explains the relatively small rates of complications such as bleeding, bile leakage, biloma, urinary leakage and urinoma. Moreover, the gradually evolving inflammation, which migrates slowly from the periphery towards the center of the ablated area, might partially explain the rarity of inflammatory complications and abscess formation seen after the use of RFA.

Conflicts of interest

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