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RESEARCH

Patterns of Histological Changes following Hepatic Electrolytic Ablation in an Ex-Vivo Perfused Model

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Abstract Electrolytic ablation (EA) destroys the liver by releasing toxic radicles and producing modifications in the local pH without increasing the tissue temperature. We assessed the histological changes produced by EA using an ex-vivo perfused model. Five porcine livers were harvested, preserved in ice and reperfused for six hours in an extracorporeal circuit using autologous normothermic blood. One hour after reperfusion EA was performed and liver biopsies collected at the end of the experiments. The main necrotic zone consisted of coagulative necrosis, sinusoidal dilatation and haemorrhage with an unusual morphological pattern. The coagulative necrosis and haemorrhage affected mainly the peripheral area of the lobule with relative sparing of the area surrounding the centrilobular vein. Contrasting with this sinusoidal dilatation appeared to be more prominent in the centrilobular area. EA produces patterns of tissue destruction that have not been observed with the more commonly used thermal techniques. Further studies should obtain more information about the influence of adjacent biliary and vascular structures so that appropriate clinical trials can be designed.

Keywords Ex-vivo · Liver · Histology · Pathology · Electrolytic ablation

Main message: The safety of EA close to major vessels derives from the lack of tissue heating specific to this technique and has great potential for the treatment of awkwardly placed liver lesions. Our study demonstrates that the unusual mechanism of tissue destruction produces histological patterns that have not been described with the more commonly used thermal techniques.
Research questions:
1) What are the clinical implications of these histologic findings?
2) What is the relationship with eventual recurrences?
3) Is there a role for electrolytic ablation close to major vessels?

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Introduction

Hepatic electrolytic ablation (EA) is an experimental technique that utilizes extreme alterations in the local pH and the production of cytotoxic gases and free radicles to achieve tissue destruction [1, 2]. The principle advantage over the more common thermal techniques of liver ablation (radiofrequency – RF - and microwaves - MW) is the lack of a significant increase of the local tissue temperature [3, 4]. This is an unusual situation with ablative techniques and means that EA has the potential to be used adjacent to major vascular and biliary structures. [2, 5–7].

Histological changes following thermal ablation have been adequately described for both RF [8–10] and MW ablation [11]. Numerous studies have not only examined the macroscopic and histological findings immediately following the energy delivery ablation but also the evolution of the resulting lesions with time [10, 11]. Results demonstrated that the formation of the thermal ablation zone is a

complex combination of energy absorption, heating and conduction and possible tissue water evaporation, condensation and movement. Furthermore, the transitional zone is of paramount importance when trying to understand and predict local recurrences following an apparently successful ablation. In this zone the vast majority of the ischemic cells undergo apoptosis but a small number may survive and eventually give rise to a local recurrence [12, 13].

As the pathophysiologic mechanisms producing tissue destruction differ between EA and the more common thermal techniques, it is reasonable to postulate that the histological effects are also likely to be different. Nevertheless EA does have the potential to be used to safely ablate areas close to important intra-hepatic structures and further information about the results following treatment of these "awkwardly" placed lesions is important [6, 14]. When planning clinical studies it is of paramount importance to evaluate the histological damage that the technique produces, particularly the completeness of necrosis and the presence of apoptotic cells. We used an ex-vivo normothermic perfused experimental liver model currently in use in our laboratories to examine these changes in more detail and also confirm that the ex-vivo model was appropriate and produced comparable results to previous live animal model studies of EA. [15–17].

Materials and Methods

Liver Procurement

Intact livers were obtained post mortem from five female domestic white pigs weighing 45–60 kg. The animals used in this study received human care and the study protocols were implemented in accordance with the United Kingdom "Guidance on the Operation of the Animals (Scientific Procedures) Act 1986". Animals were humanely sacrificed with final exsanguination during the blood-harvesting procedure performed in accordance with Home Office regulations. A pre-heparinised non-pyogenic container with 5000 units of heparin was used to collect the autologous blood. After the animals were certified dead, their livers were retrieved with a minimal warm ischemic time, perfused with Soltran solution (Baxter), transported on ice from the abattoir to the laboratory and reperfused with the autologous blood as previously described [15, 17].

Liver Ablation

A direct current (DC) generator was designed to deliver a pre-determined "dose" of coulombs at a constant current which could be varied between 1 and 100 milliAmpere (mA; coulombs = current (amperes) x time (seconds)) (ECU 100, Söring GmbH, Justus-von-Liebig-Ring 10, D-25451 Quickborn, Germany; Fig. 1). Automatic voltage adjustments between 1 and 25 V allowed the pre-determined rate of current to be delivered, regardless of alterations in hepatic parenchymal resistance during treatment. Positive (anode) and negative (cathode) electrodes, used to conduct the current, were placed within the substance of the liver. Platinum electrodes were used (Johnson and Johnson, 6 French, 2 mm diameter) with the cathode and anode electrodes 1 cm apart. Anode and cathode electrodes were identical and constructed from fine platinum wire (0.5 mm diameter), which was electrically insulated using a semi-rigid plastic sleeve. Two millimetres of uninsulated electrode were exposed at the tip [1].

After the first hour of perfusion, the applicator was inserted through the hepatic parenchyma and 100 Coulomb (C) of energy delivered at 100 mA for 17 min. Upon activation of the generator, the current flow "ramped" up to its pre-determined maximum value (milliamps) over the course of 1 min. Once had achieved a "steady state", as treatment progressed the voltage required to maintain a constant current flow was varied automatically in order to compensate for any fluctuation in the resistance between the electrodes. After the pre-determined dose of coulombs was delivered, the current returned to zero over a period of 1 min.

All pigs underwent hepatic EA and six ablations of approximately 2 cm in diameter were performed in each liver (total time was approximately 120 min). Any bleeding

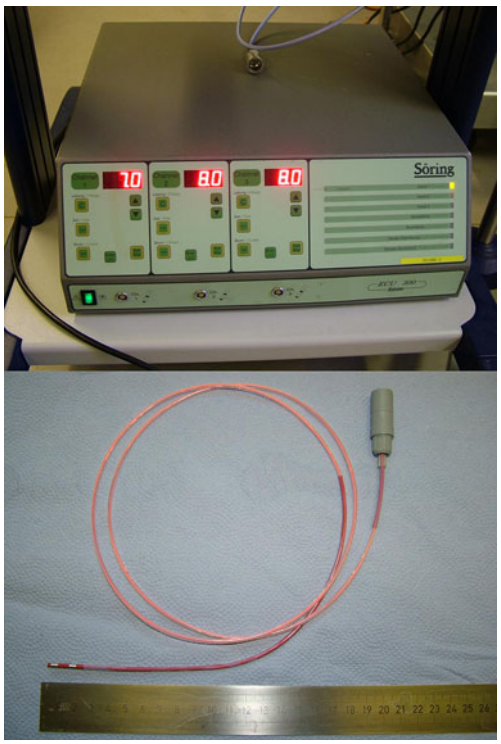

Fig. 1 DC machine (*upper panel*) and probe (*lower panel*)

from the probe insertion point was controlled by applying the probe to the surface for a further 2–3 s.

Samples Collection and Pathologic Analysis

All biopsies were taken immediately before ablation and at the end of the experiment (6th hour of perfusion). Specimens were fixed in 10 % paraformaldehyde and stored in the fridge at −4 °C overnight. The following day they were embedded in paraffin, cut into thin slices (3–5 μm) and stained with standard Haematoxylin and Eosin.

Results

All perfusions proceeded until the sixth hour without complications and eighteen lesions were successfully produced. Macroscopically the ablated area was black and sharply demarcated from the adjacent parenchyma by a whitish rim of approximately 0.5–1-mm thickness (Fig. 2). The normal parenchyma outside the surrounding rim was apparently unaffected (Fig. 2). Microscopically the black area corresponded to a necrotic zone affecting virtually all the lobules. Histological changes consisted of coagulative necrosis, sinusoidal dilatation and haemorrhages (Fig. 3). An unusual morphologic pattern was present in most specimens with the coagulative necrosis and hemorrhages affecting mainly the peripheral area of the lobule (around the portal triads and septae) with relative sparing of the central areas surrounding the centrilobular vein (Fig. 3). Contrasting with these findings sinusoidal dilatations were considerably more obvious in the centrilobular area but for both necrosis and sinusoidal dilatation changes were present to a varying degree in different areas.

Discussion

Thermal techniques of liver ablation are well established in the palliative setting and consideration is being given to their use as definitive treatment. They destroy liver tumour (and tissue) by delivering physical energy (microwave or radiofrequency) and developing high tissue temperatures through the Joule effect. The histologic lesion created, in both normal liver and cancerous tissue, roughly resemble a set of concentric spheres or ovoids centred on the tip of the cannula delivering the energy. The different histological layers develop according to the temperature gradient and

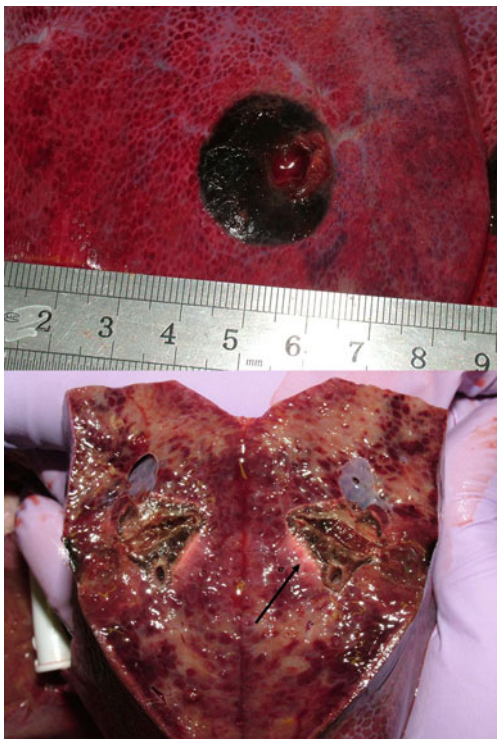

Fig. 2 Macroscopic appearance of the electrolytic ablated lesion. The colour black derives from the transformation of the haemoglobin into haemin due to the acidification of the local environment (*upper panel*) [18]. The transitional zone is usually less than 1 mm and produces a sharp demarcation between the ablated zone and the normal parenchyma (*lower panel* – black arrow)

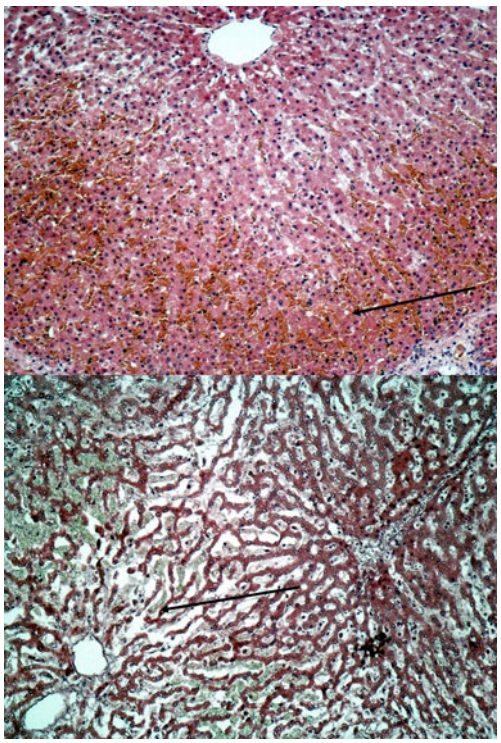

Fig. 3 Microscopic appearance of the electrolytic ablated lesion. Coagulative necrosis and hemorrhages involving the peripheral areas of the lobule around the portal triads and septae (*upper panel: Haematoxylin and Eosin 100x* – black arrow). Sinusoidal dilatations in the centrilobular area (*lower panel: Haematoxylin and Eosin 100x* – black arrow)

to a lesser extent the duration of the temperature change [10, 11]. These concentric layers consist principally of a central zone of coagulative necrosis surrounded by a peripheral transitional zone of hemorrhage, stasis, sinusoidal dilatation and apoptosis [10, 11].

Compared to thermal techniques, EA destroys tissues through extreme changes in the micro-environment and particularly the acid–base balance. The anode and cathode accumulate highly acidotic/alkaline extracellular fluid resulting in a toxic pH gradient [14, 16]. The favourable characteristics associated with non-thermal destruction of liver parenchyma [3, 4] combined with the very well demarcated and narrow transitional zone and lack of any virtually any systemic effects (despite the extreme local tissue changes in pH) [2, 18] makes this technique ideal for "awkwardly" placed lesions close to major vessels that cannot safely ablated by one of the thermal techniques [5, 14]. Despite the significant potential of the technique to date clinical evaluation as been limited probably due to the increased duration of treatment compared to the more commonly used thermal techniques [19].

Histopathologic changes obtained after EA have been already presented in previous experimental studies. Macroscopically, ellipsoidal zones of hepatic necrosis are created around the electrode tips [2]. Increased volumes of ablation may be obtained with separated [1, 5] or multiple electrodes and by the use of a Pringle manoeuvre [20]. Microscopically the central cavity corresponding to the electrode is surrounded by necrosis. The transitional zone is very narrow and extremely well demarcated with numerous microvascular thrombi [18]. Lesions around the anode characteristically desiccate and become well demarcated, and hepatocytes in this area have a pycnotic nucleus with little or absent cytoplasm [18, 21–23]. Conversely lesions surrounding the cathode attract water and show generalized oedema [18, 21–23], hepatocyte nuclear and cytoplasmic swelling and occasional disruption of the plasma membranes [18, 21]. Of significance EA produces a significantly narrower transitional zone compared to radiofrequency ablation (RFA) [19]. This may be due to the increased time required to produce the ablated area, allowing tissues to undergo a more complete and definitive necrosis. The clinical implication of a thinner transitional zone and a more complete ablation, with fewer borderline cells which may survive, is the potential to achieve proper cancer-free margins and therefore reduce local recurrences in addition to the safe use near major vascular structures.

An important limitation of our study involves the use of the ex-vivo model, especially when investigating more in details the transitional zone and the effects potentially elicited by EA in it (i.e. apoptotic changes, quantity and quality of the inflammatory reaction, cancerous cells survival and local recurrences). Unfortunately our model has only a technical follow-up period of 5 h following EA due to the ex-vivo nature of the perfusion as compared to in-vivo studies [24], therefore, it is difficult to evaluate the presence of any apoptotic cells in the transition zone due to the longer times they usually require to manifest. We initially stained some specimen looking for the apoptotic changes but found no significant changes compared to baseline values immediately after the animal's death. Therefore, we believe that a more detailed analysis of the transition zone and the long-term modifications should be properly investigated with models involving longer perfusions or in-vivo studies before drawing any definitive conclusions. Additionally, the lack of malignant cells in the ex-vivo model prevents any further extrapolation about the usefulness of the technique in the clinical setting. Again, only in-vivo experiments or clinical studies could solve this issue. Nevertheless, the ex-vivo model was able to uncover the peculiar histological modifications elicited by EA for the first time and is a valuable tool for the study of the early phases of tissue destruction while avoiding the use of live animals in these early phases of research.

Conclusions

The safety of EA close to major vessels derives from the lack of tissue heating specific to this technique and has great potential for the treatment of awkwardly placed liver lesions. Our study demonstrates that the unusual mechanism of tissue destruction produces histological patterns that have not been described with the more commonly used thermal techniques. In additional the behaviour of the liver in the ex-vivo perfused model closely mimicked that found in previously described live animal models. This finding allows preliminary studies on ablative techniques to be performed without the need for complex, expensive live animals, and without the attendant ethical considerations.

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Ashley Dennison had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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