Feasibility study using tissue as reagent for cancer therapy: endovascular ablation via thermochemistry

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PAPER

Feasibility study using tissue as reagent for cancer therapy: endovascular ablation via thermochemistry

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Abstract

The growing global burden of hepatocellular carcinoma, the poor response to chemotherapies such as sorafenib, and the inoperable status of most patients when they present clinically have led, over the last three decades, to development and application of loco-regional therapies such as ablation and embolization. Unfortunately, incomplete treatment and local recurrence are all too common with these methods. In this report, we describe a fundamentally new strategy, an image-guided embolization method employing a targeted chemical reaction to affect local biology. We demonstrate feasibility in a simple model system using an acid chloride as the electrophile in an inert carrier solvent. The reagent solution is delivered through a small catheter in the target artery. Once released, the acid chloride reacts vigorously with any water or available functional groups present such as hydroxyl or amino groups in the tissue and simultaneously generates an acidic local environment. We call this new method thermoembolization due to the exotherm that is observed in the tissues as captured by both thermocouple and infrared measurements. The *in-situ* reaction of a small volume of the electrophile delivered intra-arterially causes highly localized endovascular ablation in our model system. The ratio of coagulated tissue volume to injected material was consistently in the range of 40:1 which compares very favorably against the 1:1 ratio found in chemical ablation using direct, intratumoral ethanol injection. The largest increase in temperature observed was 24.1 °C, meaning that the thermal energy alone could be enough to coagulate tissues. The acid that is released at the same locale further enhances the denaturation observed. Taken together, these findings underscore the potential of this new approach for treating malignancies in a nonsurgical way.

Introduction

Solid tumors in the liver such as hepatocellular carcinoma (HCC) have a poor prognosis. These tumors are frequently asymptomatic, growing undetected for long periods of time [1, 2]. Due to the silent nature of the disease, patients most often present in the clinic at a point where they are inoperable. Globally, incidence of HCC is increasing and it responds poorly to systemic chemotherapy [3, 4]. However, a survival benefit has been shown using locoregional treatments for those patients who are not surgical candidates [5, 6].

Locoregional therapies are most often delivered by imaging guidance in an interventional radiology suite on an outpatient basis with either conscious sedation or in some instances general anesthesia. These include ablation and embolization, and the decision regarding which to apply depends on the particular clinical scenario [7, 8]. Smaller lesions $\leqslant$3 cm tend to be treated by ablation. Historically this was accomplished mainly by using direct injection of ethanol [9] but also to a lesser extent acetic acid [10, 11] into a tumor to chemically ablate or coagulate the tissue. Over the last two decades this has been largely replaced in developed countries [12] by thermally coagulating the tissue with either radiofrequency (RF) [13, 14] or microwave (MW) ablation [15, 16]. These methods deposit energy by a needle-type electrode or antenna that is positioned using ultrasound (US) or computed tomography (CT) guidance. These devices are placed directly in the tumor to heat it and cause thermal denaturation, effectively burning tissue *in situ*. In larger tumors and

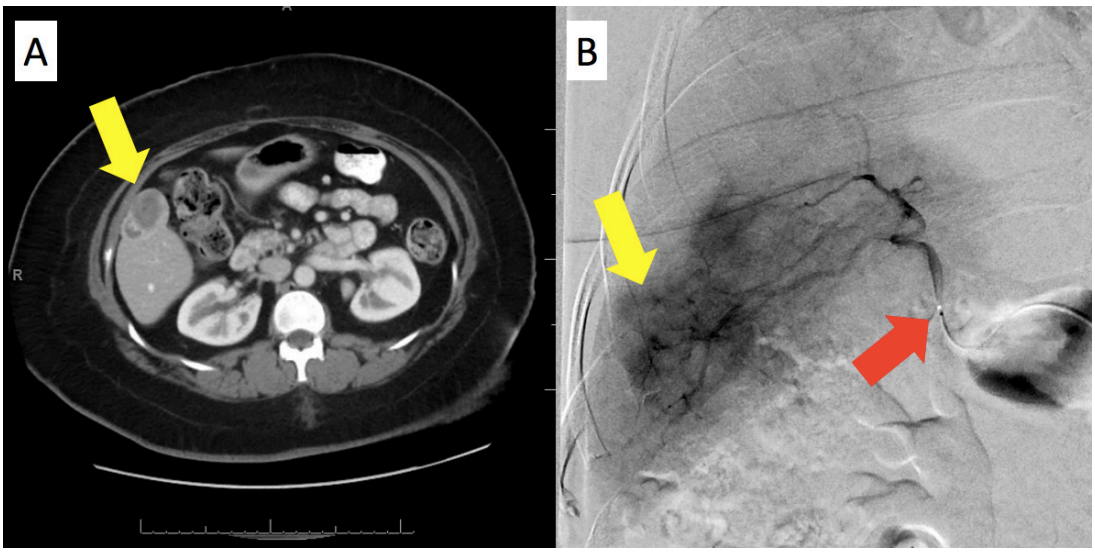

Figure 1. Typical clinical case illustrating a patient with hepatocellular carcinoma treated by transarterial chemoembolization (TACE), the current standard of care. Multiple other tumors making the patient inoperable are not depicted in this single image. (A) Computed tomography scan illustrating typical appearance of target tumor in the anterior and inferior aspect of the right lobe of the liver (yellow arrow) (B) Digital subtraction angiography demonstrating hypervascularity of the same tumor in liver (yellow arrow), manifest as a rounded, dark blush of contrast compared to surrounding normal liver. Contrast is delivered by a microcatheter (red arrow) in the artery just upstream with the tip in the right hepatic artery. The same catheter is then used to deliver therapy, which is generally either an emulsion of ethiodized oil with a drug such as doxorubicin, or drug-eluting beads loaded with doxorubicin or similar drugs. Incomplete treatment is quite common as described further in the text.

multiple tumors, RF and MW ablation outcomes drop off significantly. Thus, these cases are most frequently treated using a method called embolization. This is a fluoroscopic technique in which an intra-arterial catheter is positioned just upstream from a target tumor (figures 1(A) and (B)). Once the catheter is in place, chemotherapeutic drugs and an embolic agent are delivered to block blood flow to the tumor.

This method causes ischemia similar to a heart attack or stroke, and is commonly referred to as transarterial chemoembolization (TACE). Both ablation and embolization methods have drawbacks, however, that can lead to tumor recurrence and limit therapeutic benefit.

For ablation, a major limitation relates to the blood flow in surrounding tissues. Perfusion can lead to a heat-sink effect that prevents some of the tumor cells from reaching a lethal temperature. The larger the tumor or the closer the tumor is to a high flow vessel, the greater the probability that this will occur. Further, our knowledge of the systemic effects of hyperthermia is in its infancy and there are reports that ablation can provoke a more aggressive residual tumor response [17]. Adjacent organs may also be at risk for non-target burn injury. For embolization, relative vascularity plays an important role in how much material can be deposited into the vascular bed of the tumor. Hypervascular tumors may respond better than those which have poorly developed neovascularity, perhaps because more of the embolic agent can be delivered. Unfortunately, in treated, explanted liver specimens (diseased organs removed from liver transplant recipients) residual tumor is very common [18]. Because of these issues, combination procedures applying both ablation and embolization have been performed in an attempt to gain better control of tumors [19–21].

Opinions vary for combining TACE and ablation, and there is debate among those advocating the combination on which of these two procedures to do first. A case has been made to perform TACE first, in order to decrease blood flow to the tumor and deliver drug, and then do RF or MW ablation. The rationale is that blocking the arterial blood flow limits the heat sink effect and that it is presumably better to have a drug present already in tissues when an ablation is performed. This does not, however, account for portal venous blood blow which is by volume the dominant supply in the liver and is not treated by TACE. On the other hand, if ablation is done before embolization, blood flow around the periphery of the burn zone increases temporarily due to inflammation. The thought is that this response allows for greater delivery of embolic material at the margins if done while still actively occurring. The literature is unclear as to which approach might be best [22].

Unfortunately, doing two procedures raises additional issues. Patients having both procedures done are exposed to the twice the risk and twice the cost and additional damage is done to the liver. Further, the delay between procedures can be days to weeks, allowing time for surviving tumor cells to adapt. Thus, having an option which would be a single procedure, combining multiple synchronous modes of attack, seems a promising strategy. This is in line with Hanahan's advocacy of a simultaneous, multiple-angle 'battle-space plan' [23]. Heated ethiodized oil [24]

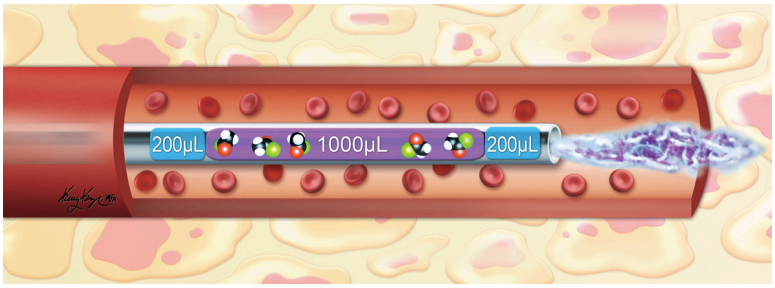

Figure 2. Depiction of concept for catheter delivery of the thermoembolic solution in the target vessel providing blood supply to the tumor. In order to prevent early reaction with water or blood inside the catheter, the solution was delivered as a bolus of the reagent dissolved in the inert solvent (purple) and sandwiched between two aliquots of the inert solvent (blue) loaded at the leading and trailing edges. Reaction of the electrophile was anticipated to begin shortly after exiting the catheter.

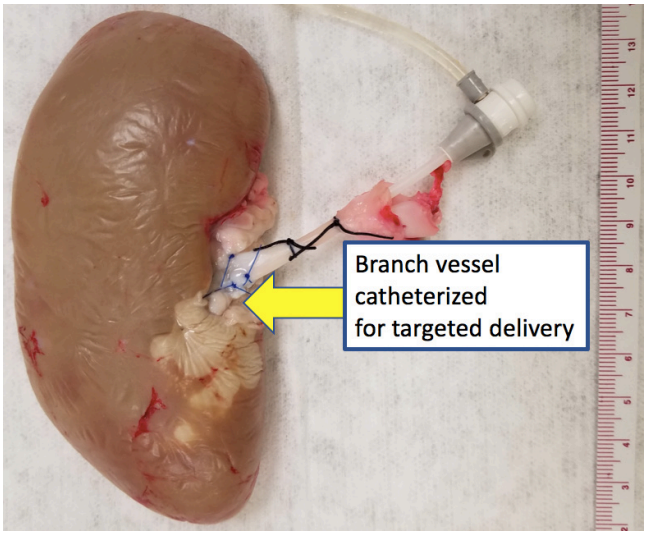

Figure 3. Experimental setup. Arising from the aorta, the origin of the main renal artery is cannulated with a sheath which is sutured snugly to prevent backflow around the sheath during infusion. Small amounts of capsular fat are present near the hilum visible as light tan areas coincidentally similar in color to the subsequent coagulation. The sheath tip was situated in a first order branch supplying one pole of the kidney in much the same manner as a catheter would be positioned in vivo for selective targeting of tissue. Thermocouples were then positioned at the untreated pole as a control, near the hilum (central region), and coaxially down the sheath for point measurements of temperature.

and hot saline [25] delivered transarterially have both been reported in an investigational setting with some degree of success but only employ a thermal component. In our new approach, we combine hyperthermia, ischemia, and chemical denaturation. In the specific example in this report we also add an additional, high-local-dose pharmacologic attack as discussed further below.

With this background, we became interested in the possibility of performing chemical reactions with imaging guidance in a biologic setting, directly in the tissues, rather than in flasks as is usually the case. In situ chemistry by direct injection into tissue has been reported previously using several reaction types [26–31] but it has never been attempted via the arterial route. The reagent or reagents would be delivered by a catheter placed in a feeding artery and delivered locally (figure 2).

Conceptually, we hypothesized that such a method would be advantageous because it could simultaneously *internally* ablate and embolize a target vascular bed in a single procedure while limiting systemic exposure and toxicity. In this report, we describe initial feasibility experiments with a simple ex vivo model system (figure 3). The kidney was chosen for these feasibility studies instead of liver tissue due to the ease of securing arterial access, simple anatomy, and availability. A branch vessel was selectively catheterized to provide controlled targeting.

We exploit the in situ exothermic hydrolysis of dichloroacetyl chloride (DCA-Cl, figure 4). We refer to this new method as thermoembolization.

Results and discussion

Infrared imaging indicated a temperature rise near the renal hilum that began almost immediately (figure 5), corresponding to the position just inside the tip of the sheath.

The temperature recorded by thermocouples in the sheath and in adjacent tissue peaked at approximately 74 s after injection. At the tip the sheath It increased an

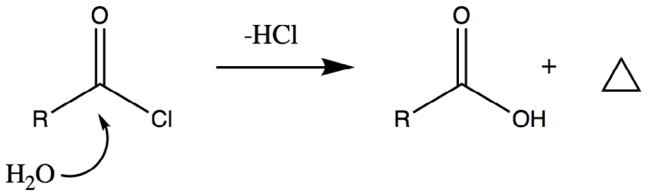

Figure 4. Aqueous hydrolysis reaction of an acid chloride as predicted in thermoembolization with water as the dominant nucleophile. Note the generation of two acidic protons from formation of the parent carboxylic acid and loss of HCl, as well as the release of substantial heat energy (−93 kJ/Mole).

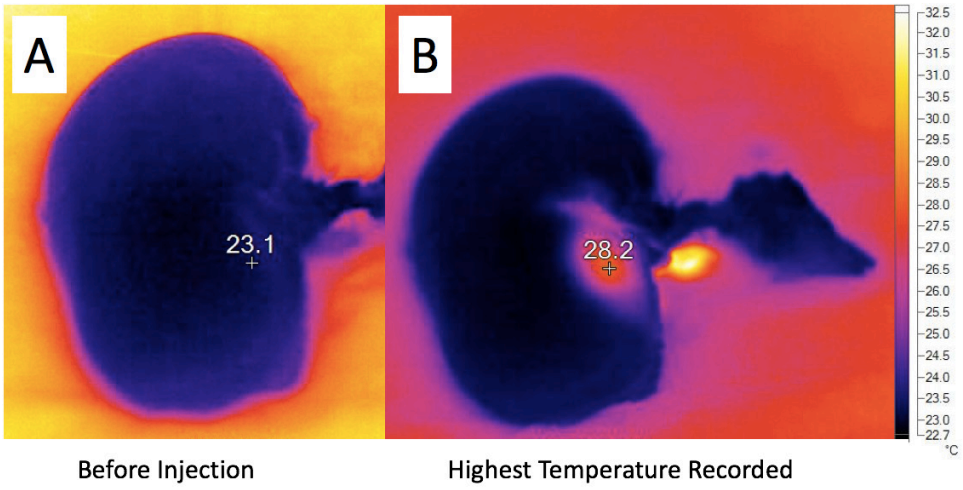

Figure 5. Infrared images of thermoembolization obtained (A) immediately before and (B) at 24 s following completion of the injection. Peak heating is observed at the hilum of the organ at the approximate location of the tip of the sheath. In this example the temperature increase at the surface by infrared thermometry measured 5.1 °C. Note that in graph below, peak temperature at thermocouple placed in this region was significantly higher deep in the tissue.

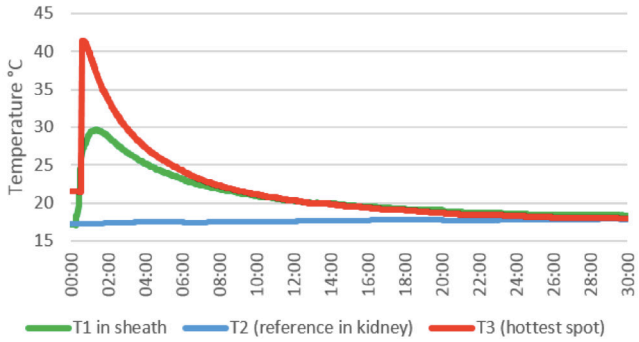

Figure 6. Representative graph of temperatures measured from baseline reference at opposite pole of kidney (blue) just within the tip of the sheath (green), and from the hottest location identified by infrared imaging (red, accounting for the higher initial temperature). Injection began at 10 s and lasted for 6 s, sampling at 1 Hz. The temperature rose abruptly with the peak at 25 °C above the baseline and gradually decayed over the 30 min duration of the experiment. Peak temperature deep in the tissue is much higher than indicated on the surface by infrared imaging and also higher than at the sheath tip.

average of 8.1 ± 3.7 °C from baseline and at the hottest point over the reference probe the temperature increased 15.7 ± 8.5 °C (figure 6, supplemental data S1 (stacks.iop.org/CSPO/4/025003/mmedia)).

Coagulation of tissue was discernable on the surface of the kidney as a pale, tan region that began to appear immediately after the injections. The damaged area enlarged somewhat overnight and became confluent (figure 7).

On average, the procedure caused coagulation of 41 ± 3.8 cm^3 tissue as measured at 24 h after injections, compared to total kidney volume of 81 ± 9.0 cm^3 (supplemental data S2). On the H&E stains of the tissue sections (figure 8 and supplemental data S3), injection of DCA-Cl caused widespread, diffuse tissue damage characterized by basophilic appearance on H&E stain, absence of red blood cells, cell disruption, contraction, and loss of cell attachment to the base-

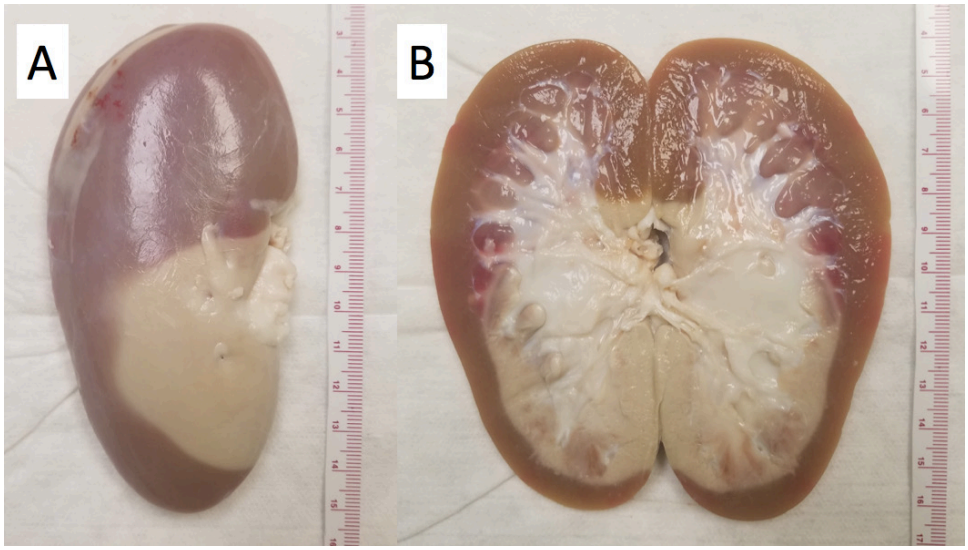

Figure 7. Gross pathology after thermoembolization with 1 ml of reagent solution showing selective coagulation of tissue resulting from the procedure. (A) Initially somewhat mottled in appearance, the pale area seen in the external view became confluent and well defined over 24 h. (B) Internally, damage is widespread and confluent as well as seen in bivalved specimen. Volume of coagulation ranged from 40% to 50% of the total volume of kidney tissue.

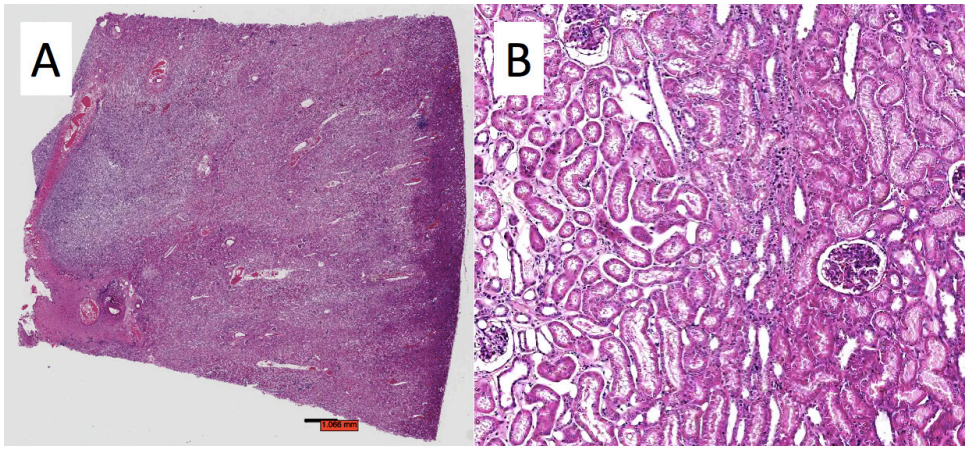

Figure 8. H&E stain of formalin fixed representative treated kidney demonstrating combined effects of heat with acidic environment in thermoembolization (A) low and (B) magnified (10×) views. Architecture of renal cortex (right, darker stain, both views) is preserved up to the transition zone. Untreated tissues are more eosinophilic and cell attachment to basement membranes is preserved. In the treated area (left side, pale blue color) the coagulation is so extensive that only portions of the underlying tissue framework are appreciable. Severe contraction and loss of cellular integrity is widespread.

ment membrane. In some cases, tissue decomposition was so extensive that it led to fracture on sectioning. In untreated areas, normal renal cortical architecture is identifiable with full/rounded glomeruli, convoluted tubules readily appreciated, and overall a more eosinophilic coloration. Tissue damage in treated areas, however, is so wide-ranging that it is more difficult to appreciate the organ framework.

An important factor in planning this study was choice of materials that could be transported via catheter. Optimal materials for delivery via this approach would most likely be liquids at room temperature or small particulate reactive solids that could be dissolved or suspended in an inert delivery vehicle, were readily available, be reactive but not pyrophoric, not generate any gases as reaction products, and have acceptable or even useful reaction products. Small molecular weight electrophiles such as acid chlorides appeared to suit our purpose.

One of the major concerns at the beginning of this study was reactivity of the electrophile. Although the hydrolysis of an acid chloride is thermodynamically quite favorable [32, 33], it was possible that the equilibrium distribution between solution and tissue could be a problem. If the electrophile did not partition from the solution into the tissues, no reaction would ensue. The only therapeutic benefit in that situation might be embolization and thus ischemia. Alternatively, from a kinetic standpoint, distribution out of the inert solvent might occur but be so slow as to be ineffective in contributing to the overall outcome. Given the relatively fast temperature increases observed, the magnitude

of the increase, the duration of the decay curve, and the extent of coagulation observed, these fears proved unfounded. This same reactivity accounts for why a small volume of mineral oil was used at the leading and trailing edges during injection and transit through the catheter. If the solution encountered any water in the catheter on either side during transit, the compound could react prematurely and the exotherm would not have occurred in the target zone. As vessels course distally in the circulation, they branch continuously into smaller and smaller size. Since surface area increases accordingly, this may also have contributed to the observed outcome.

Very shortly after injection, the treated areas invariably took on a pale, tan, mottled appearance consistent with coagulation. However, over a longer period of time, an interesting observation was noted. Treated areas lost the mottled pattern and consistently became both larger and confluent. This suggests that a significant degree of diffusion occurred, which we speculate is most likely attributable to the acid generated in situ rather than delayed thermal effects. It further hints at the potential power of this technique due to the small volume employed in the injection and the distance the damage extended from the vessels. From a clinical standpoint, it also suggests a need for greater understanding of the behavior of such materials delivered by the endovascular route. It is conceivable that with such an extensive area of involvement that collateral damage would be possible if the method were not properly utilized. Fortunately, accurate delivery through highly selective arterial catheterization under fluoroscopic guidance has been a routine matter in clinical practice for over three decades [34]. Thus, the precise targeting required for thermoembolic coagulation should be readily attainable in vivo in most cases. Further control beyond the selectivity shown in the current study should be a simple matter of altering the volume injected, the concentration of the reagent, or both.

The decision to use DCA-Cl bears further comment because of the added mode of anti-tumor activity. The hydrolysis product of this compound is dichloroacetic acid, a relatively strong acid ($pK_a = 1.26$) with an expected chemical ablative effect on that basis alone. The conjugate base dichloroacetate has significant biological activity by itself [35]. The low pK_a of the acid is such that it is essentially completely ionized at physiologic pH. In clinical use, this means that high doses of the drug over long periods of time are normally required to deliver enough to the target tissues for a therapeutic effect. Although such extensive exposure can lead to reversible systemic toxicity it should be borne in mind that the metabolic activity has been exploited on a chronic basis to successfully treat inborn errors of metabolism without excessive toxicity. It has been used as an investigational drug as a treatment for the group of rare mitochondrial diseases resulting in congenital lactic acidosis [36].

Among its many biological effects, it can be viewed as an anti-tumor drug due to its ability to function as an inhibitor of pyruvate dehydrogenase kinase. This means that abnormal aerobic glycolysis frequently seen in cancer, referred to in oncology as the Warburg effect, is impaired [37]. Tumor cells are shunted from preferential aerobic glycolysis into a more normalized metabolic program of oxidative phosphorylation. This change leads to anti-proliferative effects and apoptotic cell death in a number of types of cancer [38]. When used in thermoembolization, we predict minimal systemic exposure due to cessation of blood flow in the treated area. However, a relatively high concentration but very localized deposit of the drug would be left behind in the devascularized treatment area. This could be advantageous from a pharmacokinetic standpoint to allow diffusion at the margins into adjacent tissues.

With respect to volume of the zone of coagulation and amount of material delivered, we must make some assumptions in order to analyze the results. First, we assume that all of the electrophile reacted in a relatively short time frame. The amount delivered places an upper limit on the amount of energy released in this time if the reaction does go to completion. With 1 ml of 4 mol L^{-1} solution, this equates to administering 4 mmol of reagent. Since the exotherm from hydrolysis may be approximated at -93 kJ mol^{-1}, this equates to 372 J. If we estimate that the time for hydrolysis at 100 s, this equates to 3.7 W. If we assume that diffusion is much slower and the reaction time is therefore much longer, the power value becomes even lower. The temperature difference would be progressively less over time as well. Even at 100 s for complete reaction, such low power from an energy perspective would seem to indicate that multiple effects must be operative in order to account for the observed outcome. One possibility is that this is due to the interaction between heat energy and chemical denaturation through lowering the pH in the local environment.

Examining the volume of the territory of coagulation and relating it to total kidney volume and amount of material used underscores the ability of this new method to denature tissue in situ. Using 1 mL of embolic solution, over 40 mL of tissue was treated and ultimately coagulated, representing 50% of the total kidney volume. This is very efficient when viewed in terms of the amount of coagulation vs the volume administered, and stands in marked contrast to the ratio seen with chemical ablation using direct intratumoral ethanol injection. In that method, the volume of the alcohol is the same as the intended coagulation volume, meaning a 1:1 ratio of ethanol injected for the target volume. While chemical ablation using acetic acid is considered three-fold more efficient than ethanol, these are both quite modest in comparison to the 40:1 ratio that thermoembolization produced in this model system. Further, the toxicity of both ethanol and

acetic acid is an issue. Some invariably leaks into the systemic circulation. With embolization techniques in general, the blood flow is disrupted by definition, thus keeping the material primarily in the target region.

Among the limitations of this study, the use of an ex vivo system is the most obvious as cells were not alive and there was no blood flowing during injection. Because of this, it was only possible to assess feasibility through physical damage rather than viability. Furthermore, no tumor was present, so we were not able to study the effects with disordered vasculature that is common in solid tumors. The kidney as the target organ (as noted above, rather than liver) was used as a matter of expediency, due to both availability and to ease of establishing and maintaining vascular access. Fortunately, in these initial studies, the actual tissue used is not critical to the goal of the investigation. Thermal imaging in a volumetric manner would help to determine the actual peak temperatures. Infrared imaging is by definition a surface emission modality and the thermocouples measure temperature at only specific focal locations. With these points in mind, it is likely we did not capture the actual peak temperature in these experiments.

The study raises a number of new questions. Thermal history across the treated volume will be helpful in understanding the interplay between the hyperthermic effects and pH change. The diffusion that we observed is worthy of further investigation as well, to determine if the DCA salt is diffusing as well as acidic protons. Presumably this is true simply argued on the basis of charge balance and electrostatics but this should be verified. Intrinsic buffering capacity in tissue and degree of pH deviation could also be studied with advanced MR imaging methods such as acidoCEST [39]. Another significant question, since the body is made of approximately 70% water, is how much of the electrophile actually reacted with something else instead of reacting with water. While the amount of water present would argue in favor of hydrolysis being dominant over acylation, it is unlikely that water is the only possible nucleophile. There are many other possible nucleophiles present such as free hydroxyl groups on proteins and glycoproteins in the extracellular milieu. The acid chloride is intrinsically indiscriminate for practical purposes. It would be interesting to know what degree of covalent modification may have occurred and how this might affect the biology of the system. Dose response relationships have not been established and imaging properties will require additional interrogation. As these questions are clarified, we will be able to progress to in vivo studies of the effects of thermoembolic chemistry on biology. In vivo, an important consideration will be systemic exposure and toxicity. However, the interruption of the blood supply inherent in this technique means that there should be little or no whole-body exposure. Until tested in vivo, however, this remains conjecture. We conclude that as a strategy, thermoembolization is feasible. With multiple mechanisms of tissue devitalization available, we believe the new method has as-yet untapped potential justifying further study.

Methods

Reagents

Dichloroacetyl chloride was purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Mineral oil was obtained from a local retailer and used as supplied.

Tissues

Porcine kidneys were freshly isolated from animals used in a different protocol and IACUC approval was thus not needed for the current study. Immediately after euthanasia, harvested kidneys were perfused with 100 ml cold heparinized saline (1 IU mL^{-1} Heparin), stored at 4 °C and brought to room temperature before use. Care was taken at organ procurement to preserve the renal arterial supply and venous drainage. In each case a major branch of the renal artery supplying one pole of the kidney was cannulated by using a 5F introducer sheath (Cook Medical, Bloomington, IN). The sheath was secured with silk ties over the artery proximally to prevent leakage back around the sheath during injection.

Procedure

Injections were performed in three freshly explanted porcine kidneys. A solution of the electrophile DCA-Cl in mineral oil was freshly prepared at a concentration of 4 mol L^{-1} and 1 ml of this solution was drawn into a 3 ml syringe for immediate use. A 200 μL aliquot of mineral oil was loaded into the 5F sheath via the three-way stopcock of the side arm followed by the solution of electrophile in mineral oil and finally a second 200 μL aliquot of mineral oil. The sandwiched bolus was delivered over 5–6 s through the catheter followed by 1 mL of additional mineral oil to account for the dead volume of the sheath.

Temperature measurement

The surface temperature of the kidneys was monitored using an infrared thermal camera (Fluke Ti32, Fluke, Everett, WA). Additional monitoring was performed using a 4-Channel Data logging Thermometer (SDL200, Extech Instruments, Nashua, NH). Thermocouples (4-inch T type needle, Physitemp Instruments, Clifton, NJ) were used to record temperatures at a sampling rate of 1 Hz. Thermocouples were placed at three different locations on the kidney just proximal to the tip the sheath, at 1 cm depth as a reference in the cortex of the opposite pole of the kidney from that supplied by the cannulated artery, and at the hottest point identified by the infrared camera immediately after injection.

Tissue processing

Gross photographs of the kidney tissue were obtained before the procedure and after the procedure both before and after bivalve sectioning in the coronal plane for further assessment of the degree and distribution of coagulation in tissues. Tissues were stored overnight in a refrigerator for further processing. Coagulation zones were measured in three dimensions by longest axes in the coronal plane after sectioning and across the anterior/posterior dimension. Tissue samples at the border of the treated zone were harvested and frozen in liquid nitrogen without embedding medium. Hematoxylin and eosin staining was performed on 15 micron frozen tissue sections.

Data analysis and statistics

Temperature records were plotted and analyzed in Microsoft Excel. Volumes of kidney and ablated tissue were estimated using the formula $\pi/6 * (L \times W \times H)$ as previously described [40–42] and reported with standard deviations.

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