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Basic Research

Microwave Ablation of the Pig Growth Plate: Proof of Concept for Minimally Invasive Epiphysiodesis

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Abstract

Background Different surgical methods for epiphysiodesis of limb length discrepancy (LLD) have been described. Although these methods are variably effective, they are associated with morbidity (pain and limp) and potential complications. Microwave ablation is a less-invasive opportunity to halt growth by selectively destroying the growth plate via thermal energy to treat LLD in children.

Questions/purposes In this proof-of-concept study using an in vivo pig model, we asked: (1) What is the durability of response 2 to 4 months after microwave ablation of the tibial growth plate as measured by length and angulation of the tibia via a CT scan? (2) Was articular cartilage maintained as measured by standard histologic staining for articular cartilage viability?

Methods To develop an in vivo protocol for microwave ablation, we placed microwave antennas adjacent to the proximal tibia growth plate in the cadaveric hindlimbs of 18 3-month-old pigs. To determine the suitable time, we varied ablation from 90 to 270 seconds at 65-W power settings. After sectioning the tibia, we visually assessed for discoloration (implying growth plate destruction) that

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Ethical approval for this study was obtained from the University of Wisconsin-Madison's School of Medicine and Public Health Institutional Animal Care and Use Committee (Protocol ID: M006398-A01).

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included the central growth plate but did not encroach into the epiphysis in a manner that could disrupt the articular surface. Using this information, we then performed microwave ablation on three live female pigs (3.5 to 4 months old) to evaluate physiologic changes and durability of response. A postprocedure MRI was performed to ensure the intervention led to spatial growth plate alterations similar to that seen in cadavers. This was followed by serial CT, which was used to assess the potential effect on local bone and growth until the animals were euthanized 2 to 4 months after the procedure. We analyzed LLD, angular deformity, and bony deformity using CT scans of both tibias. The visibility of articular cartilage was compared with that of the contralateral tibia via standard histologic staining, and growth rates of the proximal tibial growth plate were compared via fluorochrome labeling.

Results Eighteen cadaveric specimens showed ablation zones across the growth plate without visual damage to the articular surface. The three live pigs did not exhibit changes in gait or require notable pain medication after the procedure. Each animal demonstrated growth plate destruction, expected limb shortening (0.8, 1.2, and 1.5 cm), and bony cavitation around the growth plate. Slight valgus bone angulation (4º, 5º, and 12º) compared with the control tibia was noted. No qualitatively observable articular cartilage damage was encountered from the histologic comparison with the contralateral tibia for articular cartilage thickness and cellular morphology.

Conclusion A microwave antenna placed into a pig's proximal tibia growth plate can slow the growth of the tibia without apparent pain and alteration of gait and function.

Clinical Relevance Further investigation and refinement of our animal model is ongoing and includes shorter ablation times and comparison of dynamic ablation (moving the antennae during the ablation) as well as static ablation of the tibia from a medial and lateral portal. These refinements and planned comparison with standard mechanical growth arrest in our pig model may lead to a similar approach to ablate growth plates in children with LLD.

Introduction

Children with projected moderate (2 to 5 cm) limb length discrepancy (LLD) at maturity can be treated with epiphysiodesis, which includes drilling of the growth plate and curettage from one or two incisions with fluoroscopic guidance [5, 8, 11]. This procedure is painful, with children often requiring up to 6 weeks of activity modification; additionally, complications such as angular deformity, incomplete arrest, bleeding, scarring, and infection have been encountered [2, 10]. Alternatively, some practitioners have used implants (transphyseal screws or modular plate and screw constructs) across the medial and lateral sides of the growth plate [1, 2]. This method has increased the cost owing to the use of implants, the possibility of implant failure, the need for implant removal, and the risk of asymmetric growth arrest.

Radiofrequency ablation (RFA) epiphysiodesis with subsequent growth arrest has been used in several animal models [6, 12-14, 16]. Although RFA has become the preferred modality in the percutaneous treatment of osteoid osteoma, it possesses inherent limitations regarding the size and scope of potential growth plate ablation secondary to variations in tissue electrical conductivity and thermal properties [17]. A newer technology that is becoming increasingly used in the destruction of tumor cells is microwave ablation [7, 15]. Microwave heating can create larger zones of injury, it acts more consistently within different tissues, and it requires shorter ablation times to achieve similar zones of tissue destruction [3].

In this proof-of-concept study using an in vivo pig model, we asked: (1) What is the durability of response 2 to 4 months after microwave ablation of the tibial growth plate as measured by length and angulation of the tibia via a CT scan? (2) Was articular cartilage maintained as measured by standard histologic staining for articular cartilage viability?

Materials and Methods

Cadaveric Methodology

In this portion of our study, and before the in vivo part of it, we wished to determine how microwave ablation could visually alter the growth plate of a pig's cadaver. The proximal tibia was chosen because it is a relatively disc-like growth plate and is very similar to the structure of the tibia in human children. We chose proximal tibia growth plates from the hindlimbs of 18 recently euthanized 3-month-old Landrace X pigs. These animals are rapidly growing at this age and usually start adolescence at 6 months old; growth usually ceases at 18 months. Pigs were positioned on a custom table, and fluoroscopic guidance was used to place drill holes for the microwave antenna and temperature probes (Fig. 1). We experimented with 12 cadaveric limbs by varying antenna location, antenna type, and duration of stimulation at a constant frequency of 2.45 GHz and power of 65 W. After each microwave ablation treatment, the proximal tibias were sectioned, and the spatial location and extent of the ablation site were studied. We looked to see that there was visual discoloration (implying growth plate destruction) that included the central growth plate but did not encroach into the epiphysis in a manner that could disrupt the articular surface.

From the study of the first 12 tibias, we chose two potential in vivo ablation protocols to trial in six cadaveric

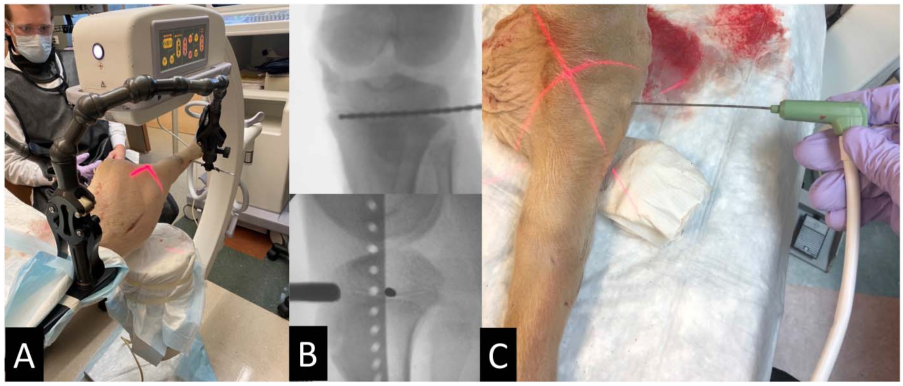

Fig. 1 Fluoroscopy (GE OEC 6800 Mini C-arm) was used to image (**A**) a pig's hindlimb to visualize proper placement of (**B**) drill tunnels and (**C**) microwave antennae through a lateral approach. Because of potential thermal damage to the soft tissues, we used a medial approach for the in vivo model.

limbs, planning to perform microwave ablation of the proximal tibia growth with a NeuWave Medical Certus 140 and PR-15 antenna (Ethicon). These two different combinations of probe location and duration were selected because the visual ablation zones affected the center of the growth plate and the visual differences did not approach the articular surface. In three randomly selected limbs, the microwave antenna was placed through the growth plate (intraphyseal) across 80% of the width of the tibia and stimulated for 140 seconds; in the other three specimens, the antenna was placed across 80% of the width of the tibia at 0.5 to 1 cm distal (metaphyseal) to the tibia growth plate and stimulated for 180 seconds. A custom radiolucent drill guide was used to ensure consistent placement.

Postablation, specimens showed large zones of radiologic and histologic tissue disruption across the growth

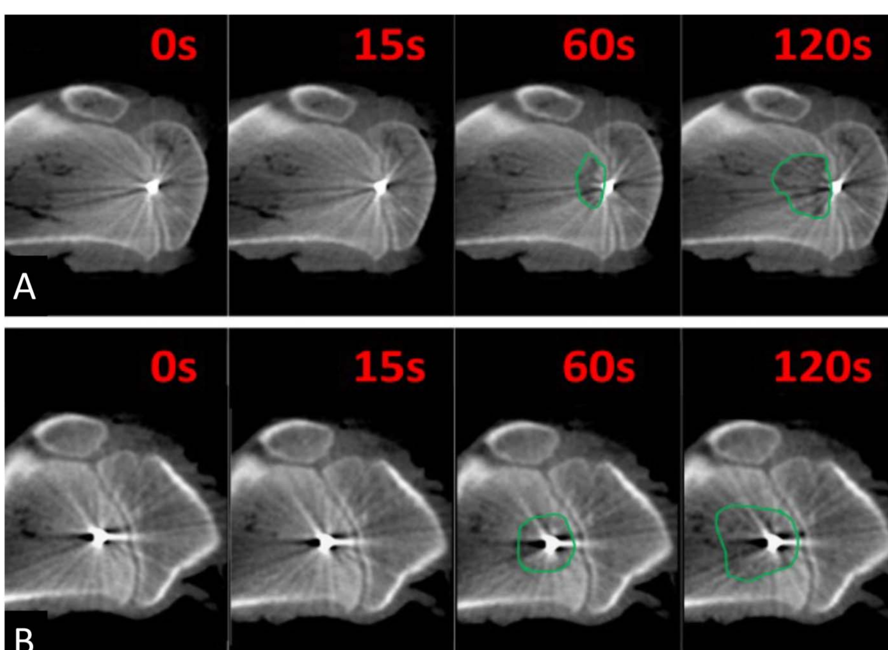

Fig. 2 Real-time ablation cone-beam sagittal CT of the (**A**) intraphyseal and (**B**) metaphyseal configurations demonstrates heat and visible gas formation (outlined in green). Red text denotes ablation times. Note the protective nature of the growth plate and general deflection of heat away from the articular cartilage.

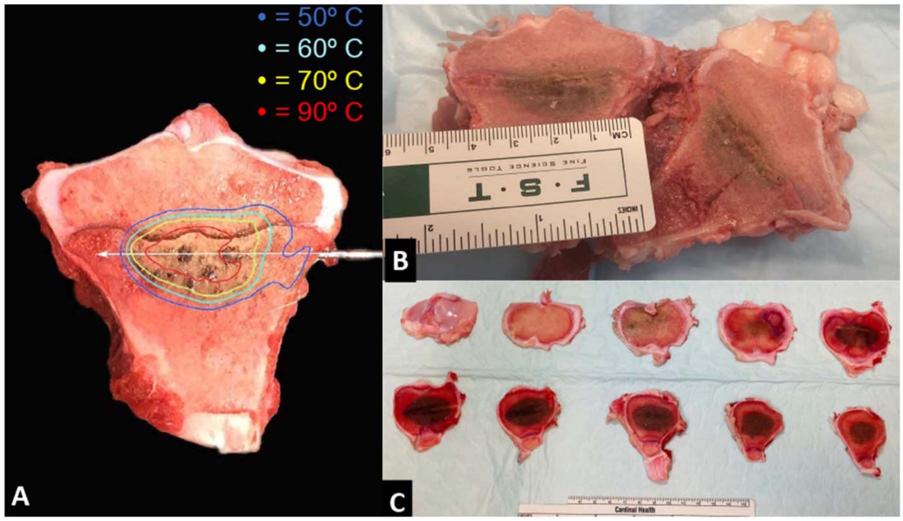

Fig. 3 (**A**) Microwave ablation of the proximal tibial growth plate in pig cadavers is shown. Infrared camera studies demonstrated real-time ablation temperatures along the growth plate well exceeding temperatures that result in cell death. (**B**) Coronal and (**C**) axial sectioning revealed consistent ablation char patterns along the growth plate.

plate with no gross damage to the articular surface when probes were placed in the growth plate (intraphyseal) as well as just distal to the growth plate (metaphyseal). For intraphyseal and metaphyseal configurations, cone-beam CT demonstrated ablation gas formation (representing greater than 30% reduction in Hounsfield units in endosteal bone) in the growth plate and metaphysis, with a propensity for heat deflection at the level of the growth plate away from the proximal region (epiphysis) and its adjacent articular cartilage (Fig. 2).

Sectioned specimens confirmed char formation across a large (> 50%) region of the growth plate with both configurations, as measured using volumetric ratios on photograph analysis. Infrared measurements of cross-sectional tibial ablation specimens demonstrated temperatures well above the estimated 60°C threshold for cell death (Fig. 3). Histologic evaluation using hematoxylin and eosin and safranin-O staining of freshly harvested pig hindlimbs (with viable cellular architecture at the time of ablation) after microwave ablation epiphysiodesis of the proximal tibias demonstrated preservation of the articular cartilage architecture with no evidence of local chondrocyte damage or abnormal proteoglycan staining with safranin-O.

In Vivo Animal Study

We performed an in vivo pilot study of microwave ablation in the proximal tibia growth plate of pigs. Three domesticated female pigs, which were of a mixed-breed Landrace-Large White-Duroc variety, approximately 3.5 to 4 months old with a mean weight of 66.3 ± 2.9 kg, were used in this study. Intraphyseal ablation was the chosen location based on our cadaveric studies because it appeared that intraphyseal ablation resulted in larger char of the growth plate. With this approach, there was visual discoloration of the cadaveric tibia that included the central growth plate but did not encroach into the epiphysis, which could disrupt the articular surface.

Description of Surgical Procedure

Pigs were induced with a combination of Telazol (Zoetis LLC) (6 mg/kg) and xylazine (2.2 mg/kg) intramuscular, and intubated and maintained on 2% to 4% isoflurane. All animals received a preoperative antibiotic dose of long-acting Excede (Zoetis LLC) (5 mg/kg intramuscular), a broad-spectrum cephalosporin, and the analgesics sustained-release buprenorphine (0.24 mg/kg subcutaneous) and meloxicam (0.4 mg/kg subcutaneous). The operative limb was prepped and draped in a sterile fashion. A small vertical stab wound incision was placed over the medial proximal tibial growth plate. Appropriate views of the proximal tibia were localized using fluoroscopy (Artis Zee X-ray System table, Siemens Healthineers). A 2-mm K-wire was placed from medial to lateral, 80% across the width of the growth plate and in the center of the growth plate on the lateral view, with care taken not to perforate the lateral cortex. A small-bore Jamshidi outer cannula was then advanced to facilitate exchange of the microwave probe (Fig. 4). A NeuWave Medical Certus 140/ PR-15 antenna was used. This antenna comprises a 15-cm, 17-gauge needle housing a C02 cooling conduit intended to

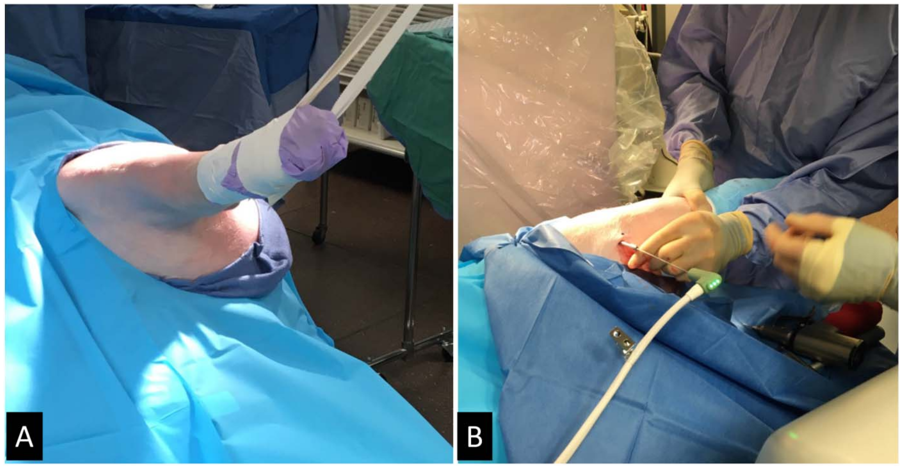

Fig. 4 A 60-kg domestic pig was anesthetized and (**A**) positioned and (**B**) underwent microwave ablation.

prevent burning of the proximal body wall. The antenna creates a direct heating zone within 2 to 3 cm of its tip.

Temperature probes were fluoroscopically placed against the medial and lateral bone surfaces at the level of the growth plate. Each animal underwent a single microwave pulse at 65 watts for 90 seconds; temperatures were recorded until a peak temperature was reached (Table 1). After microwave ablation, the wound was irrigated and closed with staples. As part of the normal recovery process, animals were observed by the veterinary team for any qualitative signs of pain or other complications. These include teeth grinding, restlessness, excessive vocalization, kicking at the belly, lameness or inability to stand (classified as mild, moderate, or severe), pain at the surgical site, or changes in posture. Video clips of the animals that showed any questionable signs of pain were reviewed with a staff veterinarian who would examine the animal. We also recorded the use of pain medicine.

Imaging Studies

To ensure the ablation protocol affected the growth plate of a living pig that was similar to what we observed in cadavers, each of the three animals underwent MRI under anesthesia within a 3-week time period. We wanted to ensure that a similar ablation pattern was seen in living tissue as in cadavers; we wanted to ensure there would be no difference between cadavers and living animals that had normal blood flow in the limb. The MRI scan (GE Discovery 3T MRI 750, GE Healthcare) included T1 and T2 sequences of the proximal tibia within 3 weeks of the ablation procedures. The time from ablation for the three pigs to undergo their single MRI was 11, 17, and 20 days. Images were studied to assess signal change from ablation in the proximal tibia and to assess for joint effusion or potential changes in the articular cartilage. One animal was euthanized early because it experienced pyometra at 56 days postoperatively, which was considered unrelated to the microwave ablation. This animal had a final CT scan at this early sacrifice, as described below.

To assess any possible changes in the bony architecture or potential fracture in the remaining two animals, we put them under a second course of anesthesia, and CT images (GE Discovery 750 CT Scanner, GE Healthcare) of the affected limbs were obtained at 61 and 65 days postoperatively. Our animal-use protocol was for animals to be euthanized at 3 months to determine the effect of microwave ablation on halting growth. As such, the two remaining animals were euthanized at 111 and 112 days from surgery. When all three animals were euthanized, the entire tibias from the control and experimental sides were harvested, dissected free, and positioned next to each other in the CT scanner to characterize any bony changes in the

Table 1. Peak temperatures after ablation

	Peak medial temperature	Peak lateral temperature
Pig 3633	37.7°C at 90 seconds	37.9°C at 90 seconds
Pig 3645	40.4°C at 90 seconds	44.6°C at 123 seconds
Pig 3646	39.9°C at 90 seconds	41.4°C at 235 seconds

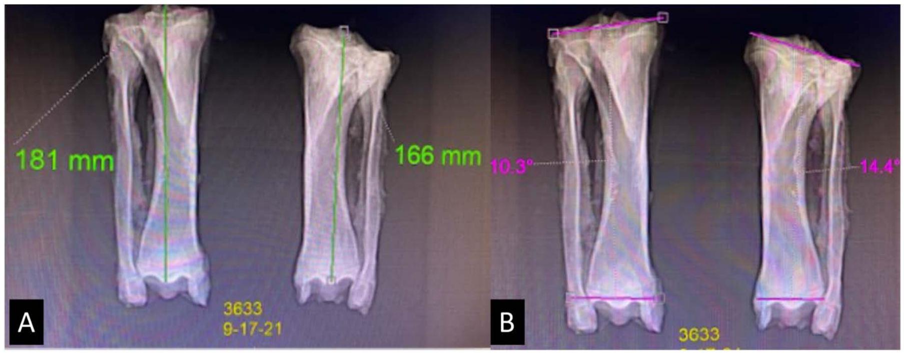

Fig. 5 CT scan at time of euthanasia allowed us to measure the differences in (**A**) length and (**B**) angulation between the control tibia (left) and experimental tibia (right). Tibial length measurements were taken proximally from between the tibial eminences and distally to the tibial plafond. A color image accompanies the online version of this article.

experimental limb and compare bone length and joint orientation between the two limbs (Fig. 5).

Histologic Analysis and Euthanasia

To measure rates of longitudinal bone growth, two fluorochromes were administered intravenously (auricular vein) to each pig at least 3 days apart over the final week before the animals were euthanized. Alizarin complexone (15 mg/kg intravenous) was given first, followed by oxytetracycline (5 mg/kg intravenous). One hour before the animals were euthanized, oxytetracycline was administered and allowed to distribute to the growth cartilage.

Euthanasia was performed according to a standard institutional protocol, and the proximal and distal tibias were dissected free and moistened with saline for CT analysis. The proximal and distal tibias were collected and sectioned. The distal tibia growth rates were included to confirm that no compensatory growth occurred after disruption of the proximal tibia. We saved 1-mm central slabs for fluorochrome measurements, and thicker slabs were placed in 10% formalin for histology. Growth rate measurements from the proximal and distal tibias were taken at 2-mm intervals across the width of each growth plate. This was to confirm that the distal tibia did not undergo compensatory growth after disruption of the proximal tibia. Measurements were taken between the two bone labels, in alignment with the chondrocytic columns of the growth plate. These measurements were averaged to give a final growth rate.

The slices saved for histologic processing were fixed in 10% NBF for 1 week and stored in 70% ethanol until they were submitted for histologic processing. One or two central slices underwent decalcification and were processed and embedded in paraffin. All tissue underwent hematoxylin and eosin and safranin O staining. Stained slides were digitally captured, and measurements of the cartilage thickness were taken starting at the surface of the superficial layer of the articular cartilage and extended down and perpendicular to the subchondral bone surface. Measurements were performed every 2 mm across the growth plate. The number of measurements taken along the articular cartilage of each experimental pig's proximal tibia were 18, 16, and 25 and along the control slices were 21, 15, and 25. The mean articular cartilage thickness ± standard deviations were calculated from pooled values of the treated versus the untreated side of all pigs.

Ethical Approval

Ethical approval for this study was obtained from the University of Wisconsin-Madison's School of Medicine and Public Health Institutional Animal Care and Use Committee (Protocol ID: M006398-A01).

Statistical Analyses

Tibia lengths and angulation measured directly after euthanasia were compared between control and experimental limbs. Bone growth rates (μm/day) and articular cartilage thickness (μm) were calculated on a per-sample basis as the mean of all measurements across each sample. Mean values of the proximal and distal tibia were calculated from pooled data ± SD of the control side versus the experimental side from all three pigs. Mean data were then compared between control and experimental groups for distal and proximal tibias using pairwise t-tests. For all comparison testing, a p value less than 0.05 was considered to indicate statistical significance.

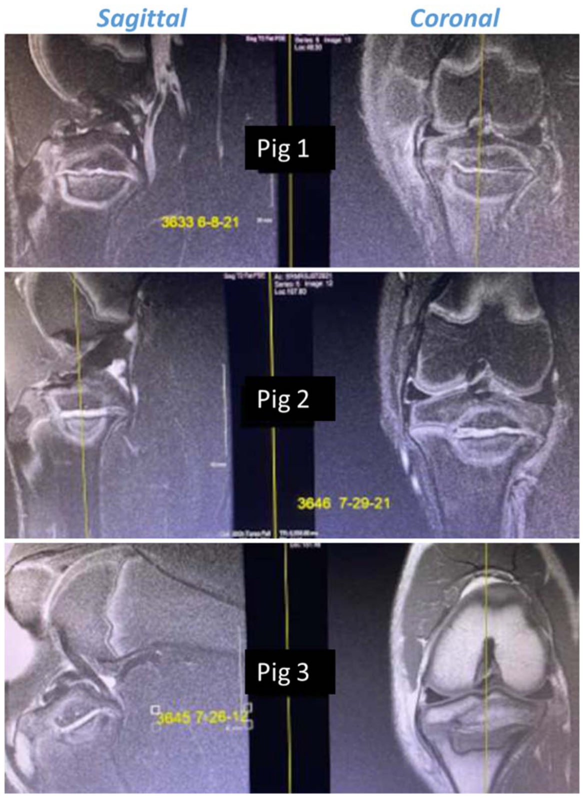

Fig. 6 MRI of live animals was performed within 3 weeks of microwave ablation of the proximal tibia growth plate. Signal changes were relatively consistent across each of the animals without any obvious articular cartilage pathology or knee effusion.

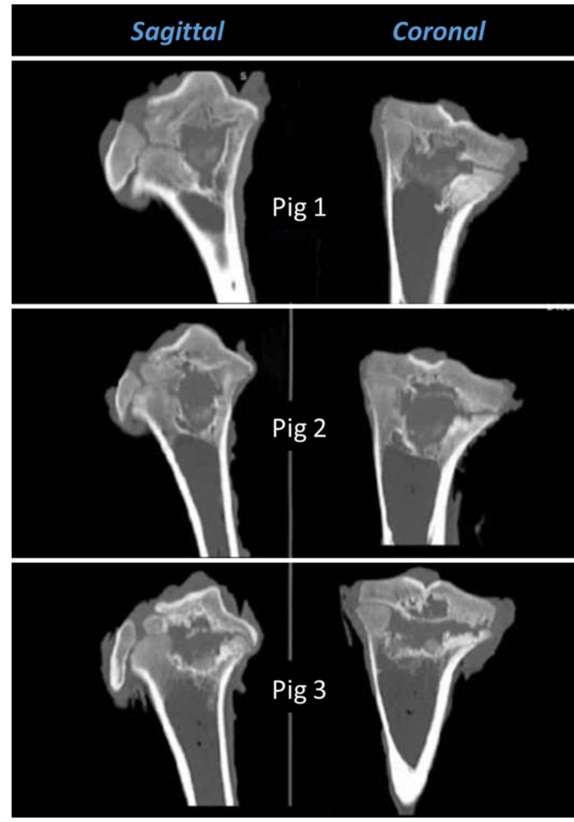

Fig. 7 This CT image shows the tibia when the animals were euthanized. Cavitary cyst formation was evident, with a predilection for the metaphyseal regions rather than the epiphyseal.

Results

Initial Animal Response to Microwave Ablation

After microwave ablation, the animals were immediately ambulatory and did not appear to have any change in their gait or activity levels. No animals had any local wound problems, redness, or swelling. As noted, one animal experienced pyometra that appeared to be unrelated to the surgical procedure. After failing to respond to two courses of antibiotics, this animal was euthanized early at 56 days postoperatively.

Imaging Results

MRI evaluation of all specimens demonstrated signal change in the growth plate with extension into the metaphysis and epiphysis. No animals demonstrated MRI findings of joint effusion, and no evidence of signal change was present in the articular cartilage (Fig. 6). CT comparison of the procedural and control limbs demonstrated bony cavitation in the metaphysis of the treated tibias (Fig. 7). No fractures or tibial plateau disruptions were noted. From the CT scans at euthanasia, we found the tibias that underwent ablation were 0.8, 1.2, and 1.5 cm shorter than the control tibias. Coronal plane angulation of 4º, 5º, and 12º of valgus was also noted (Table 2).

Histologic Results

Histologic analysis demonstrated normal articular thickness and architecture. The articular thickness on the control side was 970 ± 86 µm compared with 980 ± 78 µm on the treated side ($p = 0.89$). The cystic region on the treated side

Table 2. CT scan measurements when the animals were euthanized

	Pig 3633	Pig 3645	Pig 3646
Time to euthanasia in days	111	56	112
Shortening in cm	1.5	0.8	1.2
Valgus angulation in °	4	5	12

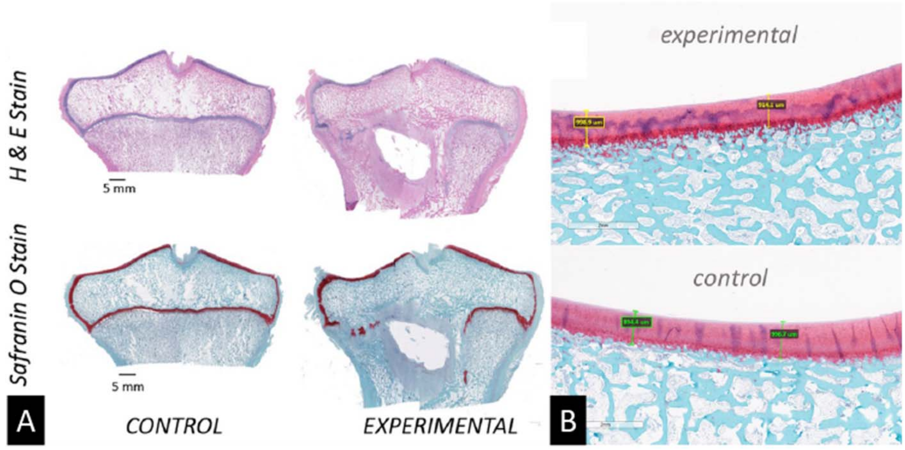

Fig. 8 (**A**) Hematoxylin and eosin and safranin O staining of the control and experimental tibia demonstrate cavitation across the physis with distinct architectural disruption. (**B**) High-power examination showed there was normal morphology and thickness of the articular cartilage.

was filled with fluid and demonstrated surrounding zones of injury and repair (Fig. 8). Comparison of growth rates of the proximal tibia between the treated tibia and control tibia rates was difficult because of the distorted architecture and obliteration of the growth plate in 40% to 70% of its width. The growth rates of the proximal tibia on the control sides were 115 ± 48 μm/day, and on the treated side (including only measurements from the growth plate that were not disrupted owing to ablation), the growth rate was 80 ± 30 μm/day (p = 0.16). There was no difference in distal tibial growth rates, with 55 ± 14 μm/day on the control side and 55 ± 24 μm/day on the treated side (p = 0.87).

Discussion

Growth arrest to correct LLD or prevent skeletal deformity after partial growth plate arrest in children is commonly done with mechanical drilling or via placement of implants across both sides of the growth plate. Each of these operative methods is invasive and painful and possesses inherent advantages and disadvantages [4, 9, 10]. Early functional return after less-invasive methods could be of substantive value because knee stiffness is a common complication after contemporary human epiphysiodesis [10]. Recent advances in minimally invasive tumor ablation with microwaves present an opportunity to use this technology to treat LLD in children. Our study sought to determine whether this technology could arrest growth in the pig tibia with a staged assessment (first in cadavers and then in a small pilot study in living pigs). From this initial study, we learned that microwave ablation can disrupt the growth plate and shorten the limb in treated animals, without changes in gait or need for supplementary pain medicine. These data have led us to begin other studies that seek to determine the ideal location, duration, and method of ablation in a large group of animals to someday use this technology to control growth in children.

Limitations

Although we believe this study demonstrates that microwave ablation can disrupt growth of the proximal tibia in a pig model, there are steps to pursue before microwave ablation could one day be considered to treat LLD in children. To realize this objective, several obstacles must be considered and overcome before translation to humans with LLD is considered. First, this is an animal model, and we must enhance its effectiveness in pigs. Because most human growth arrest procedures are performed in the tibia and femur, we must determine whether this can work in the femur of the pig, which has a similar biconcave architecture as humans. In pigs' tibias, we noted some angulation of the tibia at the time of euthanasia, which would be an unacceptable outcome in a human, and we also noted some bony cavitation with unknown importance.

Discussion of Key Findings

Regarding the angulation seen in our pilot animals, we feel this is because of a single static ablation, which led to lateral growth arrest with some continued medial growth resulting in angulation. The fact that the growth rates of the remaining physis are less than those of the control side would argue against asymmetric growth acceleration as a mechanism for deformity. We are currently comparing a dynamic approach through a single medial portal whereby the antenna ablates the lateral portion of the growth plate and is withdrawn to a medial position where this is repeated. We are also using a dual approach in which the lateral growth plate is ablated from a lateral portal and the medial growth plate is ablated via a medial portal.

Prior RFA in pigs appears to show some residual cavitation with subsequent bony bar formation at 6 months after the procedure [6]. As such, we anticipate that pigs undergoing microwave ablation would demonstrate similar bony replacement. The bony cavitation seen in these animals has not affected their function, but this needs more study. These animals were euthanized at 2 to 4 months, and we do not know the long-term outcome of these radiographic findings at maturity and whether the ablations would have resulted in a change in the shape of the articular surface or attachments of intraarticular ligaments. To mitigate the size of these lesions, we recently performed live-animal ablations with temperature probes in the bone, and we learned that much shorter times are sufficient to generate temperatures that can ablate the growth plate. Perhaps shorter times combined with multiple locations for ablation as described above will be effective and limit the size of bony cavitations. Work is also ongoing to design antennae that could be tailored to selectively ablate the unique architecture of the growth plate, thus limiting ablation to only around the growth plate and potentially less metaphyseal cavitation. Although we noted that the articular cartilage was intact in these three pilot animals, each of our proposed modifications must minimize any possible articular effects, and animals in future models must be followed to maturity.

Comparison With Other Methods of Thermal Ablation

A form of thermal epiphysiodesis in animals has been described using RFA [6, 12-14, 16]. The technology of RFA has some inherent limitations when considering its application in epiphysiodesis. RFA uses an electric current, which causes local desiccation and charring around the ablation site. This effectively creates a "shell" of char around this site and increases impedance in surrounding tissue, limiting temperature increases and the total ablation zone. Furthermore, relying on conductive heat transfer results in a slow process of ablation for RFA. Additionally, the passage of electricity through the patient's body during RFA can be a source of pain and creates the potential for an electrical arc with resultant skin burns. Microwave ablation, similar to RFA, works by heating cells to lethal endpoints. Microwaves propagate readily through many tissue types, and they generate heat primarily by agitating water molecules. The advantages of microwave ablation versus other currently available thermoablative technologies include higher temperatures, faster ablation times, and reproducible results across patients and tissue types.

Conclusion

To develop a less-invasive method to arrest growth in children, this study demonstrated that microwave ablation can be used to effectuate a reproducible zone of injury in the growth plate of cadaveric tibias from immature pigs. When applied to three living pigs, we observed similar zones of injury with concordant decreases in length of the bone because of growth plate ablation. The present pilot study has produced the groundwork for future investigations into microwave ablation as a potential method of safe, reproducible, minimally invasive, and efficacious epiphysiodesis in children with LLD. Before translation to humans, we must compare the gold standard of drill epiphysiodesis in pigs with the ideal antennae and method of microwave ablation. In these two groups, parameters to compare will include the cost, time, and radiation exposure required to perform the procedure; differences in pain and function after the procedure; and the ability to arrest growth without deformity or undue metaphyseal cavitation and with preservation of the articular health and congruity.

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