Application of High-Intensity Focused Ultrasound for the Treatment of Vascular Anomalies: An Experimental Study in a Cockscomb Model

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Key words
- high-intensity focused ultrasound (HIFU)
- vascular anomalies
- Hainanhui cockscomb

Abstract

Objective: We investigated the efficacy and safety of high-intensity focused ultrasound (HIFU) treatment in an animal model of vascular anomalies together with the energy efficiency relationship after varying exposure times and energy levels.
Materials and Methods: Hainanhui cockscombs were irradiated using a CZP therapeutic apparatus with an optimal focus over different time periods. The changes in cuticular tissue of the cockscombs and the vascular regions were analyzed by TTC dye, H&E staining, light microscopy, and Doppler ultrasound. The rectal temperatures, weight, water and food consumption of the cocks were also measured during the whole experiment.
Results: H&E staining of the tissues and combs demonstrated similar histological findings, including vessel content, and thickness of the cuticular layers (ds). There were appreciable changes to the cockscombs after doses of 2.6 w × 200 s and 3.6 w × 120 s of HIFU exposure, with 100% effectiveness and 0.00% and 37.50% irreversible damage, respectively. There were no differences with regard to water consumption, weight, rectal temperature and foraging levels monitored pre- and post-HIFU administration of the two doses.
Conclusions: HIFU exposure is effective in destroying the vascular targeted anomaly with no skin burns, bleeding, large vessel ruptures or other complications. HIFU is feasible, noninvasive and safe for the treatment of vascular anomalies, but clinical trials are necessary.

Introduction

Vascular anomalies are the most common benign tumors found in children. Clinically, many vascular anomalies require treatment because of their location, size, behavior and potential for significant complications [16,17]. Various therapeutic approaches have been described including pharmacological and physical therapies. Recently, minimally invasive therapies have been developed as alternative modalities, including laser therapy, cryotherapy, radiofrequency ablation, microwave therapy, and high-intensity focused ultrasound (HIFU), all of which deliver various kinds of energy to induce coagulative necrosis of target vascular anomalies [1,2,9,31]. Clinical applications of these modalities are increasing, and they may eventually play an important role in the treatment of vascular anomalies [11,26]. The ability to cause cell death in tissue that is distant from the ultrasound source has made HIFU attractive for development as a noninvasive surgical tool. Over the past decade, the use of HIFU has been investigated in many clinical settings and is now becoming an accepted treatment therapy in Europe and China [12,24,25].
HIFU ablation is a noninvasive modality for the treatment of localized tumors. An ultrasound beam can be focused as it passes through soft tissue. This enables the use of an external ultrasound energy source to induce thermal ablation of a tumor at a depth through intact skin. Under the guidance of real-time ultrasonographic (US) imaging, the motion of a therapeutic transducer can facilitate ablation of a three-dimensional target. The main advantages of HIFU are that it is noninvasive, conformal, and permits the ablation of large-volume tumors [5,12,22,25].
However, many issues related to HIFU treatment remain unresolved. The energy of an ultrasound beam is attenuated by its passage through tissue and, because of absorption by the tissue, only a small amount of energy penetrates to the deep tissues. A high-energy ultrasound beam, however, may cause skin burns or destroy normal tissue during its passage to the target area. Further-

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Bibliography
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Table 1 Dosage distribution of HIFU exposure.

Exposure time	Power			
	1.6 w	2.6 w	3.6 w	4.6 w
120 s	1.6 w × 120 s	2.6 w × 120 s	3.6 w × 120 s	4.6 w × 120 s
200 s	1.6 w × 200 s	2.6 w × 200 s	3.6 w × 200 s	4.6 w × 200 s

more, the small coagulation volume produced by the ultrasound beam causes the problem of low treatment throughput (treatment volume divided by treatment time). Thus, treatment times may be longer than desired and certain types of tissue may not be suitable for HIFU treatment [12,25]. A number of studies have been carried out to solve these problems, that is, to obtain sufficient energy for ultrasound penetration that will allow coagulation of tissues *in situ* while shortening the treatment times of HIFU therapy [7,24,28]. However, no current consensus exists regarding the appropriate selection of candidates, and the therapeutic efficacy of HIFU therapy in vascular anomalies cannot be reliably predicted [27]. Others have demonstrated that cockscomb is an excellent animal model for vascular anomalies [2]. We therefore investigated the use of HIFU for the treatment of vascular anomalies and vascular malformations, and compared the results with normal skin. Although a number of studies have previously evaluated the treatment of patients with benign and malignant tumors using transrectal HIFU devices, to the best of our knowledge, the efficiency of HIFU for the heating and coagulation of vascular tissue anomalies has not been evaluated. Our results represent the first report of an experimental study into the treatment of vascular anomalies and vascular malformations using HIFU therapy.

Materials and Methods

Experimental animals and tissue collection

The protocols for animal care and experimental management were approved by the Children's Hospital Scientific Committee, Chongqing Animal Research Institute. Sixty-one health Hainanhui cocks were provided by the experimental animal center of Chongqing Medical University. The Hainanhui cocks were cared for and handled in accordance with the national regulations for experimental animals. The thickness of all Hainanhui cockscombs exceeded 2 mm. The animals were randomly divided into eight groups (8 animals/group); each group received one of the following dosages: 1.6 w, 2.6 w, 3.6 w, 4.6 w HIFU, and each group was subjected to either 120 s, or 200 s exposure (**Table 1**).

Protocol for the HIFU System

A therapeutic HIFU system (Chongqing Haifu HIFU; Chongqing Haifu, Chongqing, China) was used to treat all Hainanhui cocks under real-time US guidance, as described in detail previously [30]. The full details of the ultrasound treatment procedure are available on request. After HIFU exposure, the Hainanhui cocks were monitored daily for 28 days to record foraging, water consumption, weight, and rectal temperature. Macroscopic images of external change were photographed and all Hainanhui cocks underwent pre- and posttreatment color Doppler US with a 3.5-MHz convex-array probe (Q-2000; Siemens, Erlangen, Germany). Color Doppler US was performed jointly by two radiologists (Yi Tang, Qiao Wang), and the measurements were interpreted as a consensus between three observers (Yi Tang, Qiao Wang and Shan Wang, with 20, 7, and 8 years' experience of color Doppler US, respectively). With the pulsed Doppler method, the cuticular layer and subcutaneous vascularity were evaluated as intratumoral flow signals. We noted only whether pulsatile color flow was present or absent within the hemangioma tissue. Each animal was sacrificed after 28 days, and the cockscombs were examined using electron microscopy and H&E staining.

Table 2 F score interpretation system.

Score system	Description
A	1 = no changes 0 = reddening
B	1 = no changes 0 = bubble formation or excoriation
C	1 = vacuole formation or vascular occlusion 2 = disaggregation of micrangium and collagen fibrils 3 = no changes
D	1 = fibroblast endoplasmic reticulum expansion 2 = capillary endothelium damage 3 = no changes

Observation of cell death under transmission electron microscopy

A piece of the cockscomb tissue was taken, fixed in 0.2 M cacodylate buffer/3% glutaraldehyde for 30 min, dehydrated, and embedded in Epon812 following fixation. The fixation was removed with three rinses in 0.2 M cacodylate. Samples were then postfixed in 1% OsO_4 in 0.1 M cacodylate for 1 h, rinsed once in dH_2O, dehydrated through a graded ethanol series, and embedded in epon-araldite. Thin sections were cut on a Reichert Ultracut E, picked up on copper grids, and poststained with uranyl acetate and Reynold's lead citrate. Grids were examined and observed under JEM-1200EX transmission electron microscopy (Japanese JEOL Company, Tokyo, Japan).

F score interpretation

For quantitative and integrative analysis, all subjective findings were subjected to 4 scoring systems: A, B, C, and D. Macroscopic external changes were subjectively graded as A scores, and cuticular changes under microscopy were graded as B scores. The C score defined the hypoderma changes under microscopy. The D score was determined by the electron microscopy findings. The detailed gradations are described in **Table 2**. The final F score was calculated using the formula $F = (C + D) \times A \times B$.

Statistical analysis

Statistical comparisons were carried out using SPSS software for Windows (SPSS, Inc., Chicago, IL, USA). For comparisons, a rank test was carried out to analyze F scores between two groups. The incidence rate of HIFU exposure was investigated using Pearson's χ^2 test or Fisher's exact test for between-group differences. Results were considered statistically significant at $p < 0.05$.

Results

Coagulation necrosis induced by HIFU

In the preliminary comb histology experiment, the trends over the range of power widths (1.6 – 5.0 w) and exposure time (20 – 200 s) were evaluated. In the preliminary experiment (**Fig. 1**), the cockscombs were treated with two dosages of 2.6 w × 200 s

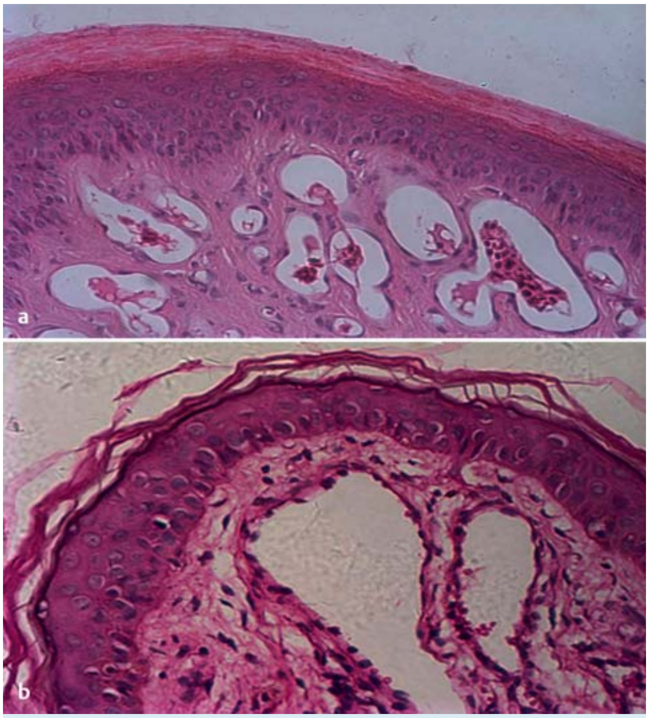

Fig. 1 a and **b** Photomicrographs of vascular histopathology from different groups, including vascular anomalies and Hainanhui cockscomb (H&E × 200). Human vascular anomalies tissue samples and Hainanhui cockscomb were subjected to section, H&E stain then quantitively analysis under microscopy was undertaken to correlate their vascularity patterns with pathological nature in an attempt to differentiate between vascular anomalies and Hainanhui cockscomb. Similar vessel content were seen in the two different tissues. **a** Humanvascular anomalies, **b** Hainanhui cockscomb tissue. Representative fields are shown above.

and 3.6 w × 120 s to monitor the effectiveness of HIFU. Histological analyses were carried out by microscopy and color Doppler flow imaging. Findings at histopathological analysis showed the presence of a solitary focus of induced coagulation necrosis that measured 6–14 mm in diameter (range: 8.9 mm ± 2.2) (**Fig. 2 d, e**). In some animals, a contiguous area of cell death was observed. In some animals in which a focus of enhancement coagulation necrosis was identified on post-ablation contrast-enhanced images, an island of viable coagulation necrosis surrounded vessels that traversed the focus. These results indicate that HIFU was an effective treatment in an animal model of vascular anomalies.

Quantitative analysis of the effectiveness of HIFU and the optimum dose

Relative effective rates and irreversible damage rates were assessed using color Doppler flow imaging assay and histological analysis. There were appreciable changes in the macroscopy patterns of the cockscomb and results with permanent decrescence. The results indicated that the F score for the combs were zero for all groups, which indicates a cuticular injury observable under histopathological examination. The highest F score for 2.6 w × 200 s and 3.6 w × 120 s HIFU exposure within the irradiated region recorded immediately after HIFU exposure was 4 (the highest achievable score is 6), with definite induced vascular lamina coagulation injury as verified by histopathologic examination, which means that HIFU exposure was effective

Table 3 F scores of the two groups.

Groups	F scores of all samples	p
2.6 w × 200 s	0, 2, 2, 2, 3, 3, 4, 4	p = 0.0216 [rank test]
3.6 w × 120 s	0, 0, 0, 0, 2, 2, 2, 3	

Table 4 Effective rate of HIFU exposure administered to subepidermis vascular lamina of cockscomb *in vivo*.

Groups	Efficacy	Inefficacy	Total	Effective rate (%)
2.6 w × 200 s	8	0	8	100
3.6 w × 120 s	8	0	8	100
total	16	0	16	100

Table 5 Incidence of HIFU exposure contributing to irreversible injury of subepidermal vascular lamina of cockscomb *in vivo*.

Groups	Incidence (n)	No incidence (n)	Total	p value
2.6 w × 200 s	0	8	8	p = 0.10 [Fisher's exact test]
3.6 w × 120 s	3	5	8	

(**Tables 3** and **4**). According to the posttreatment color Doppler US evaluation, both decreased vessel and blood flow patterns were observed for 2.6 w × 200 s and 3.6 w × 120 s exposure. The doses of 2.6 w × 200 s and 3.6 w × 120 s produced the highest coagulation necrosis and the dose of 2.6 w × 200 s induced the lowest irreversible injury rates in the comb tissues after HIFU exposure (**Table 5**). **Fig. 2** shows the representative pattern observed for 2.6 w × 200 s dosage of HIFU exposure. According to our results, the effective power and duration of HIFU were found to be 2.6 w × 200 s.

Safety assessment

To evaluate possible complications related to HIFU treatment, complications such as skin burn, bleeding, and large vessel rupture were monitored daily for 1 week. Living statural item, including water consumption, weight, rectal temperature and foraging levels were recorded for each Hainanhui cock by three observers (K. Q. L., C. B. J., F. L. X.). In all Hainanhui cocks, neither the complications nor living statural item for the 2.6 w × 200 s and 3.6 w × 120 s groups showed statistically significant differences to controls (no HIFU exposure) during this period. There were also no differences between the pre- and post-interventional exposure to HIFU.

Discussion

As a form of primary tumor of the microvasculature, vascular anomalies are characterized by excessive angiogenesis, followed by regression of the newly formed vessels. Up to 20% of vascular anomaly cases require intervention, especially when the vascular anomalies interfere with normal functions (e.g., breathing, vision, hearing, eating, voiding, and movement), produce serious disfigurement that is unlikely to resolve on its own, or contribute

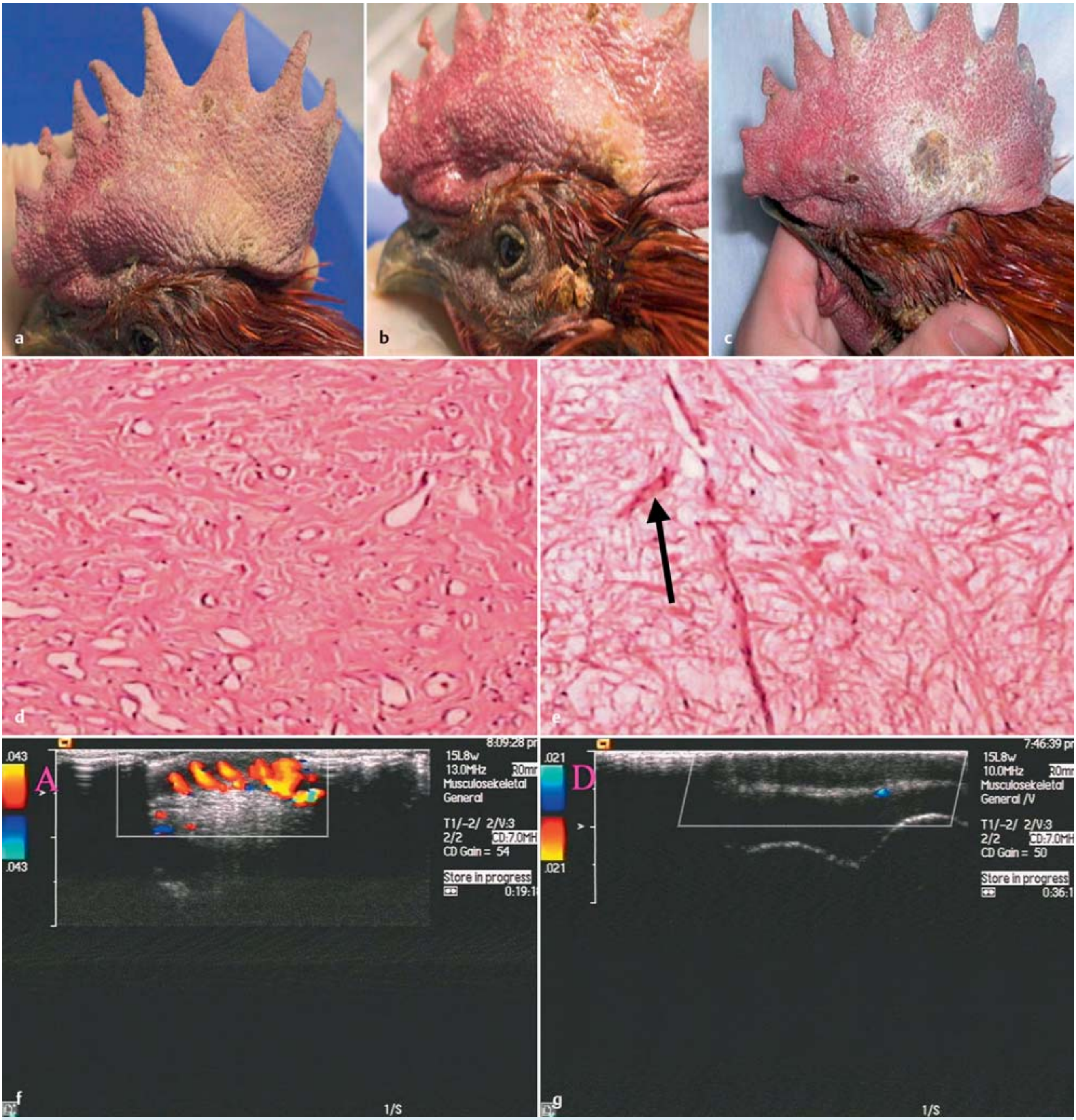

Fig. 2 a to **g** Changes of Hainanhui cockscomb under 2.6 w × 200 s HIFU exposure. Macroscopic images of Hainanhui cockscomb obtained before high-intensity focused ultrasound exposure (**a**), immediately after high-intensity focused ultrasound exposure (**b**) and 28 days after high-intensity focused ultrasound exposure (**c**). Ultrasound beam was directed into cockscomb surface from above. Formation of white individual ellipsoid regions of coagulation necrosis was observed (**c**). No macroscopic differences were observed among the A, B, C groups. Unit of ruler is centimeters. Photomicrographs of Hainanhui cockscomb tissue obtained before high-intensity focused ultrasound exposure (**d**, H & E × 100) and after high-intensity focused ultrasound exposure (**e**, H & E × 100, vascular occlusion was shown as arrow indicated). Transverse color Doppler sonograms display the sonographic characteristics of blood supply before high-intensity focused ultrasound exposure. Image displays here (**f**) showed hypervascularity color signal that is markedly increased over that of baseline images, on which little to no signal was observed. Sonographic image of hypovascular pattern (**g**) was observed 28 days after HIFU exposure.

to multiple other problems (intractable bleeding, ulceration, infection, pain, coagulation defects, heart failure, etc.) [8,19].
Although numerous therapeutic strategies have been utilized, such as radiotherapy, sclerosing agents, corticosteroid hormone injection [3,23], the reported effects were low and varied dramatically. As a noninvasive treatment, HIFU is a competitive alternative to conventional surgical treatment [20]. Clinical trials have evaluated the use of HIFU therapy for tumors of the breast, liver, prostate (benign prostatic hyperplasia and cancer), bladder, and kidney [6,13,20]. Ultrasound energy deposited in the

target tumor induces coagulation necrosis. Both the thermal and the cavitation effects induced by ultrasound energy result in tissue damage. HIFU does not require the insertion of an applicator into the target tissue, and an extracorporeal source can be used to treat large-volume tumors under real-time imaging guidance [4,15,29].

Other reports have also confirmed the use of cockscombs as an ideal model for the study of vascular anomalies [14,18,21]. The vascular occlusion effect of HIFU has also been studied by others [10]. In our study, we performed *in vivo* experiments to investigate the efficacy of HIFU for the treatment of vascular anomalies. Several different dosages combined with different exposure times were measured to choose the optimal exposure with the greatest efficacy and safety. The mean extent of coagulation necrosis created after 2.6 w × 200 s and 3.6 w × 120 s in the *in vivo* experiments tended to be the same and these parameters offered the greatest safety. The target tissue is coagulated more effectively with the higher dosage, when the dosage was increased from 2.6 w to 3.6 w. From these findings, we believe that the dosage has a greater effect than time. The experimental evidence in our study also showed that HIFU could destroy stratum vascular tissue at fixed-point, kill tumor cells and restrain the vascular proliferation at last induction of permanent occlusion with no damage to the epidermis of the cockscomb. This indicates that HIFU was effective and feasible in the treatment of vascular anomalies. Histological sections for the two exposures were stained with hematoxylin-eosin, and showed significant levels of necrosis and no hemorrhage in the tumors treated with HIFU when compared with sections of control (untreated) tissues. The results presented here clearly demonstrate the feasibility and beneficial effects of HIFU exposure to provide therapy in situations not amenable to conventional surgery or salvage therapy. The advantages of focused ultrasound therapy include the ability to focus on the area of therapy with remarkably sharp margins. The device can also be adjusted according to the depth of focus extending even to a depth of 3 or 4 cm. The procedure can be repeated as many times as required. Furthermore, focused ultrasound ablation does not preclude the use of other therapeutic options, including subsequent surgery. According to our clinical experience, clinical side effects in early trials involved damage to tissue outside the target area.

The limitation of our study is that the imaging equipment that we used to assess the follow-up results, compared with current state-of-the-art equipment, was not good enough to enable us to determine the effectiveness of the treatment. This will be improved in a future study. In our study, follow-up examination showed no differences in water consumption, weight, rectal temperature and foraging levels with cocks not exposed to HIFU. This suggests that HIFU did not influence the quality of life of cocks.

In summary, the results from our study are very encouraging. Our preliminary experience suggests that HIFU is safe and feasible for the treatment of patients with certain types of vascular lesion, but clinical trials are necessary. Our data here offers an initial step towards a greater utilization of HIFU for the treatment of vascular anomalies. A randomized clinical trial, however, is essential to determine the future role of this treatment in patients. Absent of mechanisms demonstration during the exposure will and ultimately require further investigations, such as real-time measurements of temperature increases with thermocouples and ultrastructural histological analysis of induced alterations. Further evaluation is also needed to investigate the treatment efficiency in different tissues.

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Conflict of Interest: None

References

1 *Alster TS, Railan D.* Laser treatment of vascular birthmarks. J Craniofac Surg 2006; 17: 720 – 723
2 *Chang CJ, Sun CH, Liaw LH, Berns MW, Nelson JS.* In vitro and in vivo photosensitizing capabilities of 5-ALA versus photofrin in vascular endothelial cells. Lasers Surg Med 1999; 24: 178 – 186
3 *Delesalle F, Staumont D, Houmany MA, Breviere GM, Piette F.* Pulse methylprednisolone therapy for threatening periocular haemangiomas of infancy. Acta Derm Venereol 2006; 86: 429 – 432
4 *Divkovic GW, Liebler M, Braun K, Dreyer T, Huber PE, Jenne JW.* Thermal properties and changes of acoustic parameters in an egg white phantom during heating and coagulation by high intensity focused ultrasound. Ultrasound Med Biol 2007; 33: 981 – 986
5 *Ebbini ES, Yao H, Shrestha A.* Dual-mode ultrasound phased arrays for image-guided surgery. Ultrason Imaging 2006; 28: 65 – 82
6 *Fujikura K, Otsuka R, Kalisz A, Ketterling JA, Jin Z, Sciacca RR, Marboe CC, Wang J, Muratore R, Feleppa EJ, Homma S.* Effects of ultrasonic exposure parameters on myocardial lesions induced by high-intensity focused ultrasound. J Ultrasound Med 2006; 25: 1375 – 1386
7 *Haar GT, Coussios C.* High intensity focused ultrasound: physical principles and devices. Int J Hyperthermia 2007; 23: 89 – 104
8 *Haggstrom AN, Drolet BA, Baselga E, Chamlin SL, Garzon MC, Horii KA, Lucky AW, Mancini AJ, Metry DW, Newell B, Nopper AJ, Frieden IJ.* Prospective study of infantile hemangiomas: clinical characteristics predicting complications and treatment. Pediatrics 2006; 118: 882 – 887
9 *Hemangioma Investigator Group, Haggstrom AN, Drolet BA, Baselga E, Chamlin SL, Garzon MC, Horii KA, Lucky AW, Mancini AJ, Metry DW, Newell B, Nopper AJ, Frieden IJ.* Prospective study of infantile hemangiomas: demographic, prenatal, and perinatal characteristics. J Pediatr 2007; 150: 291 – 294
10 *Ichihara M, Sasaki K, Umemura S, Kushima M, Okai T.* Blood flow occlusion via ultrasound image-guided high-intensity focused ultrasound and its effect on tissue perfusion. Ultrasound Med Biol 2007; 33: 452 – 459
11 *Ichizuka K, Ando S, Ichihara M, Ishikawa T, Uchiyama N, Sasaki K, Umemura S, Matsuoka R, Sekizawa A, Okai T, Akabane T, Kushima M.* Application of high-intensity focused ultrasound for umbilical artery occlusion in a rabbit model. Ultrasound Obstet Gynecol 2007; 30: 47 – 51
12 *Kennedy JE, ter Haar GR, Cranston D.* High intensity focused ultrasound: surgery of the future? Br J Radiol 2003; 76: 590 – 599
13 *Kennedy JE, Wu F, ter Haar GR, Gleeson FV, Phillips RR, Middleton MR, Cranston D.* High-intensity focused ultrasound for the treatment of liver tumours. Ultrasonics 2004; 42: 931 – 935
14 *Kitajima S, Liu E, Morimoto M, Koike T, Yu Y, Watanabe T, Imagawa S, Fan J.* Transgenic rabbits with increased VEGF expression develop hemangiomas in the liver: a new model for Kasabach-Merritt syndrome. Lab Invest 2005; 85: 1517 – 1527
15 *Li F, Feng R, Zhang Q, Bai J, Wang Z.* Estimation of HIFU induced lesions in vitro: numerical simulation and experiment. Ultrasonics 2006; 44 (Suppl 1): e337 – e340
16 *Marler JJ, Mulliken JB.* Vascular anomalies: classification, diagnosis, and natural history. Facial Plast Surg Clin North Am 2001; 9: 495 – 504
17 *Mulliken JB, Glowacki J.* Hemangiomas and vascular malformations in infants and children: a classification based on endothelial characteristics. Plast Reconstr Surg 1982; 69: 412 – 422
18 *Peng Q, Liu W, Tang Y, Yu S.* The establishment of the hemangioma model in nude mouse. J Pediatr Surg 2005; 40: 1167 – 1172
19 *Puxeddu R, Berlucchi M, Ledda GP, Parodo G, Farina D, Nicolai P.* Lobular capillary hemangioma of the nasal cavity: a retrospective study on 40 patients. Am J Rhinol 2006; 20: 480 – 484

20 *Randal J.* High-intensity focused ultrasound makes its debut. J Natl Cancer Inst 2002; 94: 962 – 964
21 *Ritter EJ.* The chicken comb and wattle as an experimental model for the therapy of hemangiomas. Preliminary laser studies. Life Sci 1966; 5: 1903 – 1910
22 *Sasaki K, Medan MS, Azuma T, Kawabata K, Shimoda M, Umemura S.* Effect of echo-guided high-intensity focused ultrasound ablation on localized experimental tumors. J Vet Med Sci 2006; 68: 1069 – 1074
23 *Sciarretta V, Pasquini E, Farneti G, Frank G, Mazzatenta D, Calbucci F.* Endoscopic sinus surgery for the treatment of vascular tumors. Am J Rhinol 2006; 20: 426 – 431
24 *Takegami K, Kaneko Y, Watanabe T, Watanabe S, Maruyama T, Matsumoto Y, Nagawa H.* Heating and coagulation volume obtained with high-intensity focused ultrasound therapy: comparison of perflutren protein-type A microspheres and MRX-133 in rabbits. Radiology 2005; 237: 132 – 136
25 *ter Haar G.* High intensity ultrasound. Semin Laparosc Surg 2001; 8: 77 – 89
26 *Vaezy S, Zderic V, Karmy-Jones R, Jurkovich GJ, Cornejo C, Martin RW.* Hemostasis and sealing of air leaks in the lung using high-intensity focused ultrasound. J Trauma 2007; 62: 1390 – 1395
27 *Vaezy S, Zderic V.* Hemorrhage control using high intensity focused ultrasound. Int J Hyperthermia 2007; 23: 203 – 211
28 *Wang Z, Bai J, Li F, Du Y, Wen S, Hu K, Xu G, Ma P, Yin N, Chen W, Wu F, Feng R.* Study of a “biological focal region” of high-intensity focused ultrasound. Ultrasound Med Biol 2003; 29: 749 – 754
29 *Wu F, Wang ZB, Cao YD, Xu ZL, Zhou Q, Zhu H, Chen WZ.* Heat fixation of cancer cells ablated with high-intensity-focused ultrasound in patients with breast cancer. Am J Surg 2006; 192: 179 – 184
30 *Wu F, Wang ZB, Chen WZ, Wang W, Gui Y, Zhang M, Zheng G, Zhou Y, Xu G, Li M, Zhang C, Ye H, Feng R.* Extracorporeal high intensity focused ultrasound ablation in the treatment of 1038 patients with solid carcinomas in China: an overview. Ultrason Sonochem 2004; 11: 149 – 154
31 *Yu S, Liu WY, Tang YM, Wei T.* An experimental study on the mechanism of therapy for hemangioma with glucocorticoid. Zhonghua Yi Xue Za Zhi 2005; 85: 1121 – 1124