Potential Effects of Therapeutic Ultrasound on the Healing of Immediate and Delayed Sciatic Nerve Anastomosis in Dogs

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Abstract

The objective of this study was to determine the potential effects of ultrasonic therapy in stimulating and accelerating the healing of immediate and delayed anastomosed sciatic nerve. Twenty-four dogs divided into four groups and three animals from each group were euthanised on the 30th and 60th days post anastomosis (PA). The sciatic nerve from each animal was collected for further studies. Motor and sensory nerve tests of the TIA group showed a response on the 4th and 8th weeks and in the TDA group on the 3rd and 7th weeks PA respectively. The histopathology of the TIA group showed moderate numbers of vacuolated and degenerated nerves as well as moderate perineural fibrosis on the 30th day. These became scanty on the 60th day PA. TDA Group showed few vacuolated and degenerated nerves with minimal perineural fibrosis on 30th day PA. These became scanty on the 60th day PA. In conclusion, there was accelerated healing and regeneration of the anastomosed sciatic nerves in the immediate and delayed ultrasonically treated animals compared to the control groups. Potential effects of therapeutic ultrasound on the healing of immediate and delayed sciatic nerve anastomosis in dogs.

Keywords: Anastomosis, Dogs, healing, Sciatic Nerve, Ultrasonic Therapy

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1. Introduction

Peripheral nerve injury remains a major cause of functional morbidity involving major nerve trunks in 35% of cases [1], their prognosis remains disappointingly poor [2, 3]. Peripheral nerve trauma is therefore a major cause of morbidity and carries a high cost in the healthcare [4]. Various methods have been applied to accelerate peripheral nerve regeneration and decrease the complications of delayed healing. Therapeutic effect of ultrasound waves as physiotherapy and its effectiveness in the treatment of many pathological cases in human as well as in animal cases has been reported. Ultrasonic waves may (by thermal and non thermal effects) accelerate the metabolic rate, increase circulation and extensibility of soft tissues [5]. The aim of this study therefore was to determine the potential effects of ultrasonic therapy in stimulating and accelerating the healing of immediate and delayed anastomosed sciatic nerve.

2. Materials and Methods

Twenty Four adult (2.5 years old) cross bred male dogs weighing 10-15 kg were used in this study. The animals were adapted in special cages for two weeks, and kept under similar conditions. The animals were each dewormed using a single subcutaneous dose of ivermectin 200µg\Kg (Ivomec Drench, USA), and then randomly divided into four groups. The groups containing six animals each was designed as 1. Treated immediate anastomosis (TIA), 2. Control immediate anastomosis (CIA), 3. Treated delayed anastomosis (TDA), and 4. Control delayed anastomosis (CDA). The experimental protocols, animal ethics and animal welfare were approved by the Animal Care and Use Committee (VETBAG/12.2.08/Surg 7), faculty of Veterinary Medicine, Baghdad University.

2.1 Anesthesia and Surgical Protocols

All the animals were given a single intramuscular (IM) of 0.02 mg/kg of atropine sulphate ((United States Pharmacopeia (USP) as a preanesthetic. Fifteen minutes later a single dose IM of mixed 15 mg/kg ketamine hydrochloride (USP) and 5mg/kg BW of xylazine hydrochloride (Ceva. Germany) as induction agents were given [6]. General anesthesia was maintained with 2% halothane/L oxygen. The animal was positioned laterally and the lower limb was prepared aseptically. The surgical preparation of the operated limb was extended up to the midline of the back and down to the distal third of the tibia. Surgical skin incision about 15 cm long was

done on the imaginary line extending from the great trochanter to the lateral surface of the patella on the right femur. Subcutaneous tissue was separated to expose the fascia lata, which was then sharply severed followed by bluntly dissecting and cranial reflecting of vastus lateralis muscle. The biceps femoris muscle was reflected caudally to expose the sciatic nerve which is covered by fatty tissues. The nerve was then separated from the surrounding tissues. The sciatic nerve was transected at the length of 1cm. post subcutaneous branches using blade number 15. After the accomplishment of the anastomosis according to experimental procedure, the fascia lata was closed with 2-0 vicryl simple continuous suturing, and the skin was closed by 2-0 vicryl subcutaneous suturing (Vicryl; Ethicon, UK) [7].

2.2 Sciatic Nerve Anastomosis and Ultrasound Therapy

2.2.1 Immediate Anastomosis (IA)

Sciatic nerve was transected in all animals and approximation for end-to-end of the immediately epineurial was sutured using 6/0 silk simple interrupted suturing. The operation region was then lavaged with sterile normal saline, and the fascia lata and the skin were sutured accordingly. Systemic antibiotics of Procaine Pencillin 22000 I.U /Kg BW and Streptomycin l0 mg/Kg BW were given half an hour before the operation and repeated every 12 hours for four days. The animals in TIA group were exposed to UST on the second day PA with 1watt/cm^2 for 10 minutes daily for seven days using sonodyne® machine (Oxfordshire, England) for thermal treatment after applying coupling media on the lateral and medial aspects of the operated limb. Animals in CIA group were left without UST. Three animals were euthanised from each of TIA and CIA groups on the 30th and 60th day PA, and autopsies were obtained for histopathology.

2.2.2 Delayed Anastomosis (DA)

Sciatic nerve was transected and left without anastomosis and then the wound was closed. Seven days later, the wound was reopened surgically to expose the sciatic nerve, and the transected sciatic nerve ends were anastomosed. The animals in TDA group were exposed to UST on the 8th day post transecting with 1watt/cm^2 for 10 minutes daily for seven days using sonodyne® machine after applying coupling media on the lateral and medial aspects of the operated limb. Animals in CDA group were left without UST. Three animals were euthanised

from each TDA and CDA groups on the 30th and 60th day post anastomosis. Autopsies were obtained for histopathological evaluations. The procedure was repeated as in TIA group.

2.2.3 Clinical Assessments

Daily clinical monitoring and weekly assessments were recorded until the 30th and 60th day PA for all animals. Clinical assessments on wound healing and motor nerve tests, which involved type of walk, knuckling, muscle force contraction, were carried out; as well as sensory nerve tests that involved toe spreading, lateral aspect leg sense, toe pinch and toe prick.

2.2.3. Histopathological Examinations

Two cm segment of the sciatic nerve, which involved the anastomotic line, was harvested; the nerve segments were fixed on hard and mesh plates to keep the nerve tissue from extending, and immediately preserved in 10% neutral buffered formalin. The tissue specimens were processed an ordinary dehydration, trimming and embedding in paraffin. Longitudinal sections of six microns thickness were prepared using microtome (Leica RM2255, Germany) and then the sections were stained by hematoxylin and eosin (H and E) [8]. The slides were examined under light microscopy (Olympus BH-2 Polarized Light Microscope, Japan) for histopathological evaluations. Degree of sciatic nerve healing at the line of anastomosis was graded on a scale from 1 to 5. Grade 1: Mild vacuolation, degeneration, and minimum perineural fibrosis (less than 25%), grade 2: Moderate vacuolation, degeneration, number of Schwann cells and perineural fibrosis (25-50%). Grade 3: Moderate vacuolation, degeneration, number of Schwann cells and mild perineural fibrosis, grade 4: Moderate vacuolation, degeneration, normal number of Schwann cells and prominent perineural fibrosis, grade 5: Severe vacuolation, degeneration, number of Schwann cells and mild perineural fibrosis (50-75%).

3. Results

3.1 Clinical Assessments

Daily clinical assessments of the TIA group showed that the swelling at the operation site persisted for 4-5 days, and the animals tried standing on the dorsal side of the paw, which

have severe to moderate ulcers up to the 6th day PA. In the TDA group, after the anastomosis, the swelling at the site of operation disappeared after treatment by ultrasonic waves by day three, and the ulcers on the dorsal surface of fetlock joint was healed by the 4th day PA. In the control groups, the swelling at the site of operation persisted for 4-5 days, and the animals stood on the dorsal side of the paw and there was only a slight improvement of the ulcers at the end of the 8th day of operation.

Regarding motor tests, the animals in TIA group showed the crushed, crowed and normal walking at the end of weeks 3, 5 and 6 PA respectively. Knuckling disappeared and strong muscle force contraction showed on the 4th week PA. Sensory nerve responses to tests of toe spread, lateral aspect leg sense, toe pinch and toe prick were all present on weeks 7, 8 6 and 8 PA, respectively. The motor nerve tests in the TDA group showed the crushed, crowed and normal walking on weeks 2, 3 and 4 PA respectively. Moreover, knuckling disappeared and MFC appeared on the 3th week PA. Sensory nerve tests of toe spread, lateral aspect leg sense, toe pinch and toe prick were present by weeks 6, 7, 7 and 7 PA respectively. The animals in control groups (CIA and CDA) showed the crushed, crowed and normal walking at end of weeks 4, 5 and 7 PA respectively. Knuckling disappeared and MFC appeared on the 7th week PA. The sensory nerve tests showed toe spread on the 8th week and there was no sensory nerve response to lateral aspect leg sense and toe pinch; and toe prick were present up to the end of the experiment (Table I).

Table I: Weekly assessments of motor and sensory nerve tests of all animals

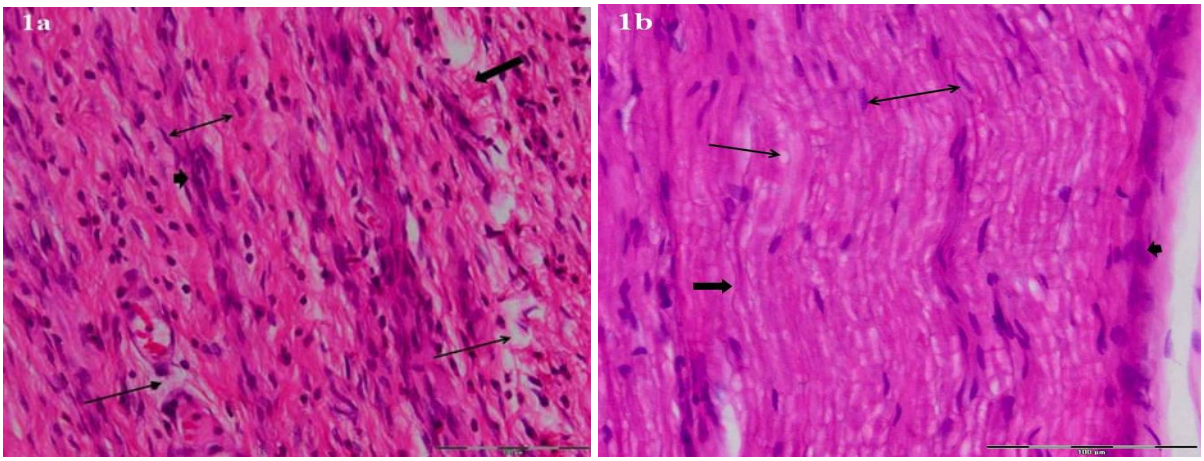
Test/ animal groups	TIA	TDA	Control (C1A, CDA)
	Time (Weeks)		
Motor Nerve Tests			
1. Type of walk			
Crushed	3	2	4
Crowd	5	3	5
Normal	6	4	7
2. Knuckling			
Sever	2	10 days	3
Mild	3	2	5
Normal	4	3	7
3. Muscle Force Contraction (MFC)			
Weak	2	1	3
Moderate	3	2	5
Strong	4	3	7
Sensory Nerve Test			
1. Toe Spread			
Absent	6	5	7
Present	7	6	8
2. Lateral Aspect Leg Sense (LAS)			
Absent	7	6	No Sense
Present	8	7	No Sense
3. Toe Pinch			
Absent	7	6	No Sense
Present	6	7	No Sense
4. Toe Prick			
Absent	7	6	No Sense
Present	8	7	No Sense

3.2 Histopathological Findings

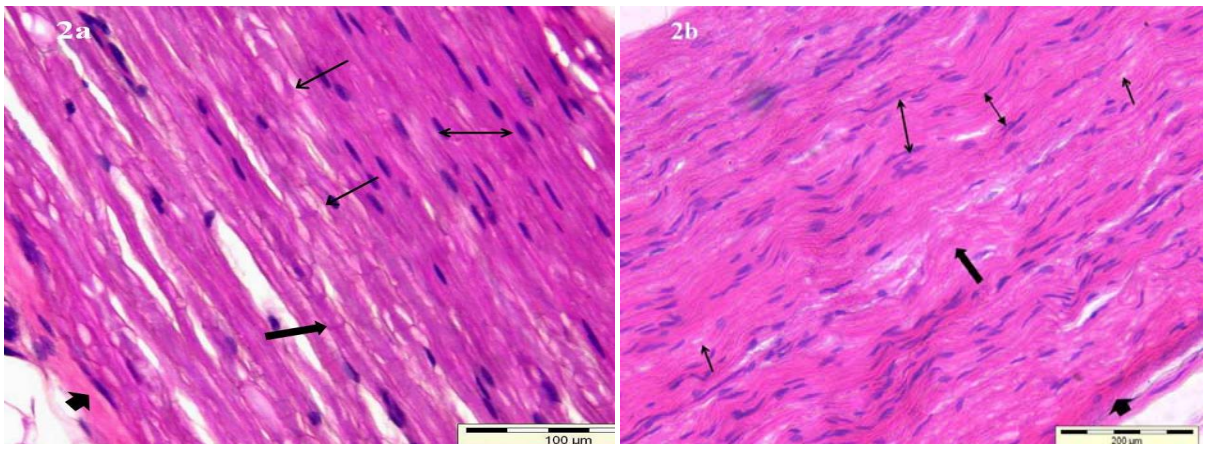
Microscopical examination of sciatic nerve sections of TIA group showed moderate vacuolated, degenerated nerve fibers, number of Schwann cells as well as mild perineural fibrosis as a grade 3 on the 30th day PA. On the other hand, moderate vacuolation, degeneration, number of Schwann cells and perineural fibrosis as a grade 2 on the 60th day PA (Figs. 1a&b).

The slide sections of TDA group showed moderate vacuolated, degenerated nerve fibers, high number of Schwann cells with moderate perineural fibrosis as a grade 2 on the 30th day PA. Mild to occasional vacuolated and degenerated nerve fibers, normal number of Schwann cells

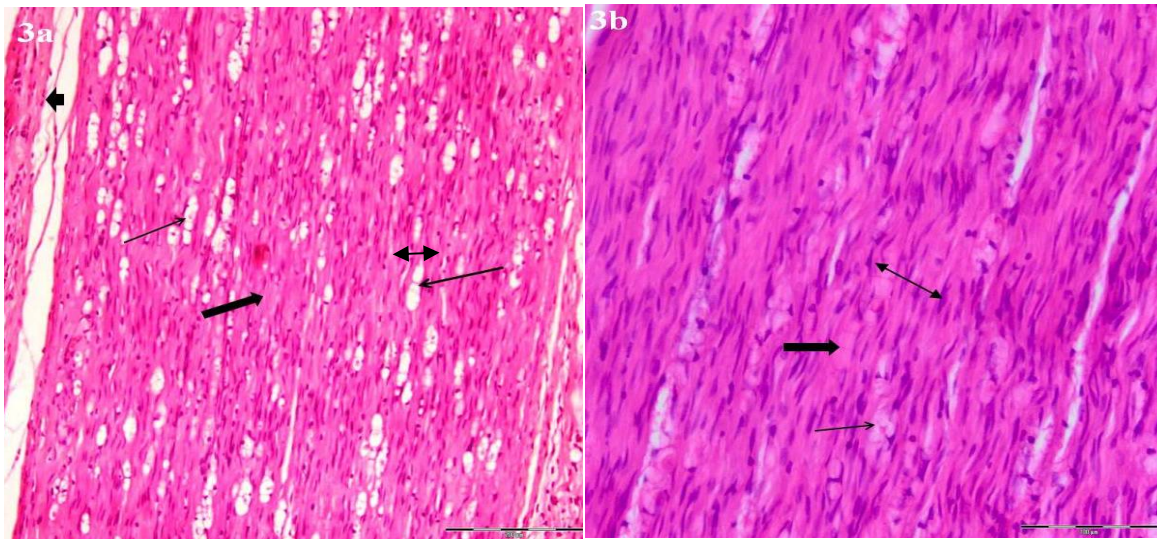
with minimal perineural fibrosis as a grade 1 were seen on the 60th day PA (Figs. 2a&b). Control groups (CIA and CDA) showed severe vacuolation, degeneration, number of Schwann cells and mild perineural fibrosis of sciatic nerve as a grade 5 on the 30th day PA (Figs. 3a&b). Moderate vacuolation, degeneration, normal number of Schwann cells and prominent perineural fibrosis as a grade 4 were seen on the 60th day PA (Figs. 4a&b).



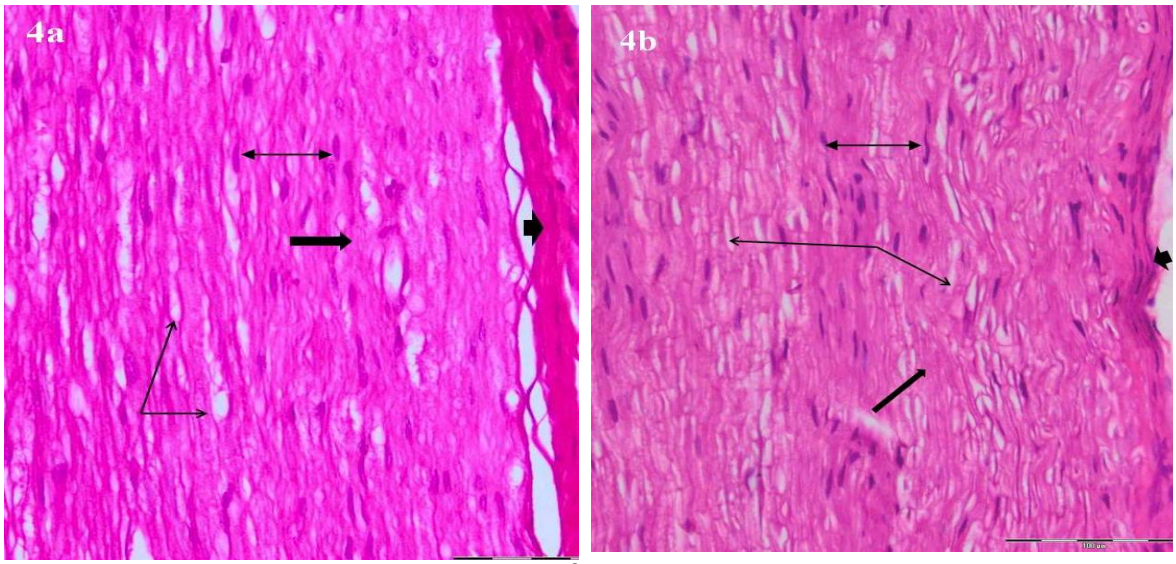
Figs 1) Histopathological photographs of anastomosed sciatic nerve in TIA group showing. 1a) moderate vacuolated (thin arrows), degenerated nerve fibers (thick arrow), number of Schwann cells (double arrow heads) and mild perineural fibrosis (arrow head) on day 30 PA. 1b). Scanty of vacuolated (thin arrow) and degenerative nerve fibers (thick arrow), (double heads arrow) with moderate perineural fibrosis (arrow head) on day 60 PA, H&E X 20.



Figs 2) Histopathological photographs of anastomosed sciatic nerve in TDA group showing. 2a) Moderate numbers of vacuolated (thin arrows), degenerative of the nerve fibers (thick arrow), number of Schwann cells (double arrow heads) and moderate periueural fibrosis (arrow head) on day 30 PA. 2b). Mild to occasional vacuolated (thin arrow), degenerated nerve fibers (thick arrow), normal number of Schwann cells (double heads arrow) with minimal perineural fibrosis (arrow head) on day 60 PA, H&E X20.



Figs 3) Histopathological photographs of anastomosd sciatic nerve in CIA group showing. 3a) Severe vacuolation (thin arrows), degenerative of the nerve fibers (thick arrow), number of Schwann cells (double arrow heads) and moderate perineura fibrosis (arrow head) on day 30 PA. 3b). Moderate vacuolated (thin arrow) degenerative nerve fibers (thick arrow), number of Schwann cells (double arrow heads) with prominent perineural fibrosis (arrow head) on day 60 PA, H&E X20.



Figs 4) Histopathological photographs of anastomosed sciatic nerve in CDA group showing: 4a) Severe vacuolation (thin arrow), degenerative of the nerve fibers (thick arrow), number of Schwann cells (double arrow heads) and moderate perineural fibrosis (arrow head) on day 30 PA. 4b). Moderate vacuolated (thin arrow) degenerative nerve fibers (thick arrow), number of Schwann cells (double arrow heads) with prominent perineural fibrosis (arrow head) on day 60 PA, H&E X20.

4. Discussion

Treatment of peripheral nerves injuries has always constituted an important medical problem, although recovery does eventually occur in most cases. Therapeutic effect of ultrasound as physiotherapy especially on tissues of high collagen content has been reported [5]. Since the peripheral nerves contain collagen, it is more responsive to treatment with ultrasound. Therefore, in this study, ultrasound was used to help patients resolve various symptoms associated with the healing process. The results of this study showed that the swelling, wounds and ulcers were minimized and the animals bears weight on the operated limbs in the ultrasonic waves treated groups using early compared to other groups. These results were similar to the observations made by other researchers [2,9]. These results were also consistent with previous work, which reported that the pain reduction might be due to the thermal and non thermal effects of the ultrasound which control the pain, by altering the transmission or perception of nerve conductive velocity, and increasing tissue temperature or modulating the inflammatory process [10]. Controlled heating can produce desirable effects, which include pain relief, decrease in joint stiffness and increased local blood flow [11]. The therapeutic effect of ultrasound on many phases of healing process also involves its non thermal properties to facilitate the process of inflammation and then healing. Ultrasound beam in the tissue has been shown to increase intracellular calcium, macrophage responsiveness of skin and cell permeability and mast cell degranulation causing the release of arachidonic acid. Arachidonic acid is a precursor of prostaglandins and leukotreines, both of which act as inflammatory mediators. Increasing the activities of these cells by ultrasound therapy is certainly pro-inflammatory rather than anti-inflammatory [12-13]. The thermal effect of ultrasound might affect vasodilatation in the exposed area, leading to increased infiltration of the inflammatory cells and other essential elements from blood circulation to the lesion site, and at the same time removing dead tissues and waste products. Therefore, ultrasound acts as a promoter of the inflammatory phase and stimulator of the proliferative phase of inflammation [14].

The results of the present study showed that the motors' sensory nerves of TDA group had earlier recovery than the TIA and the control groups. This result concurs with the finding, which reported that the thermal and non thermal effects of the ultrasound might alter the transmission or perception of nerve conductive velocity by increasing tissue temperature and

modulating the inflammatory process [10]. In this study, the clinical manifestations of knuckling disappeared and MFC appeared in TDA group early compared to TIA and control groups. This is an indication that there was growth and regeneration of some axons, which carry impulses to the distal part of the nerve. This was supported by previous report that ultrasound might accelerate axonal regeneration [15-16].

The histopathological results of the longitudinal sciatic nerve sections in this study demonstrated that the vacuolated and degenerated nerve fibers were present as wallerian degeneration, characterized by degeneration and regeneration processes at the same time [17]. Treated delay anastomosis group showed accelerated regeneration and healing of the sciatic nerve and there was obvious decrease in the number of vacuolated, degenerated nerve fibers as well as decrease in the perineural fibrosis, which might be due to the biostimulatory effect of ultrasound at the site of injury. These results were consistent with previous findings on the effects of physiotherapy on peripheral nerve [18-19]. The role of the laser might be to affect singlet oxygen that stimulated the redox activity in the respiratory chain, thus enhancing homeostasis and increasing Ca^{+} release from the mitochondria to the cytoplasm, together with increasing ATP production in the mitochondria. This in turn activates Na^{+}/K^{+} ATPase and other ion carriers, thereby increasing cell activation.

The histopathological results of this study showed an increase in the collagen fiber in the epineural and perineural of the sciatic nerve in the anatomosis line in the TDA. Therefore, the treated delay anastomosis group showed faster and better regeneration than the treated immediate anastomosis group. These results might be due to the high content of collagen deposited in nerve fibers. The results of the present study were consistent with earlier reports that tissues with higher protein content absorbed UST greater extent [5, 20-21]. The best absorbing tissues in terms of clinical practice are those with high collagen content such as ligament, tendon, fascia, joint capsule and scar tissue [20]. The predominant use of therapeutic ultrasound is in relation to tissue repair and soft tissue lesion management, where the evidence supports its application in the inflammatory, proliferative and remodeling phases [11]. In conclusion, our study showed that there was accelerated healing and regeneration of the anastomosed sciatic nerves in delayed and ultrasonically treated animals.

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