Establishing *C. elegans* as a Model for Studying the Bioeffects of Ultrasound

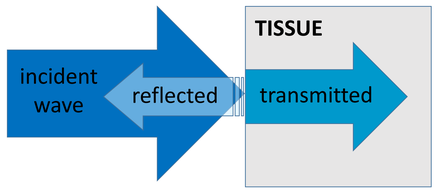
Louise M. Steele, Ph.D.[1], Troy J. Kotsch[1], Catherine A. Legge[2], and Delores J. Smith[3]
[1]Department of Biological Sciences, [2]Radiologic & Imaging Sciences Program, and [3]School of Nursing
Kent State University at Salem, 2491 State Route 45 South, Salem, Ohio 44460 · LSteeL11@kent.edu

Abstract

Ultrasound is frequently used in medicine for diagnostic imaging and therapeutic procedures. Although there is no evidence of deleterious effects from diagnostic ultrasound in humans[1]; research in flies[2,3], mice[4,5], and chicks[6] has suggested that various doses of ultrasound can affect mortality, birth weight, and learning in those species. In amoebae and cultured cells, ultrasound exposure led to cavitation, release of reactive oxygen species (ROS), and apoptosis. We **developed methods to expose *C. elegans* to therapeutic ultrasound** and found that worms exhibited dose-dependent **reductions in movement, fecundity, and survival**. We hypothesized that ultrasound may have caused inertial **cavitation** (formation and collapse of gas-filled spaces) as observed in other species' tissues[7-11]. Indeed, the effects were prevented by polyvinyl alcohol, which is known to minimize cavitation[12]. Using a genetic approach, we also looked for evidence of other mechanisms of damage. Exposed *sod-2;sod-3*, *clk-1*, and *ced-3* mutants exhibited the same changes in mobility and fecundity that exposed N2 worms did. Thus, cavitation may have masked ROS release, and apoptosis may have been insufficient to mitigate the tissue damage.

Introduction

During ultrasound procedures, interactions occur between the sound waves and the tissues in their path. **Changes that occur in the tissues are considered "bioeffects"**[14,15].



The **objective of this work was to establish *C. elegans* as model for studying bioeffects**. Further research in animals is worthwhile:

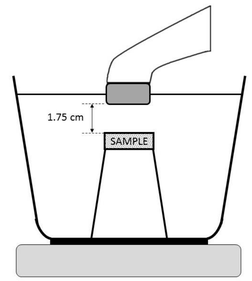
- The output and availability of ultrasound machines have increased[16,17].
- The mechanisms of some therapeutic procedures are incompletely understood.
- Ultrasound is becoming an important tool in biomedical research[18,19].

These results provide a foundation for characterizing the effects of lower-intensity ultrasound at the cellular and molecular levels.

Methods & Results

Insonating Worms

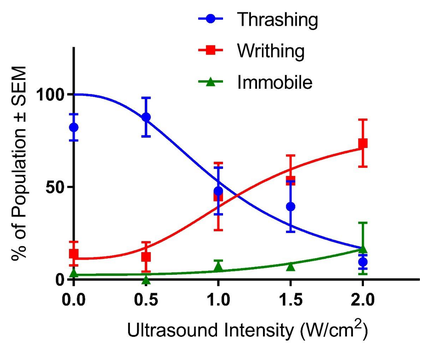
C. elegans were suspended in sterile S. Basal buffer and sealed in 35-mm Petri plates (without agar) using plastic wrap, avoiding air bubbles. The plates were immersed in a large volume of S. Basal and **insonated at various intensities at 1 MHz at 50% duty for 5 minutes.** The collimating therapeutic ultrasound probe was moved continuously to minimize heating and to give uniform exposure.



Mobility Assays

Immediately after insonation, worm movement was observed in S. Basal.

As the ultrasound intensity was increased, fewer worms were **thrashing** normally, and an increased number were moving in a slow, irregular pattern we termed "**writhing**". At higher intensities, some worms became **immobile**.



Fecundity Assays

Writhing adults were transferred to NGM-OP50 plates. The total **numbers of offspring** they produced were counted and recorded.

At the low end of the range of intensities tested, worms showed little or no change in fecundity. As exposure increased, fecundity dropped dramatically.

Survival Assays

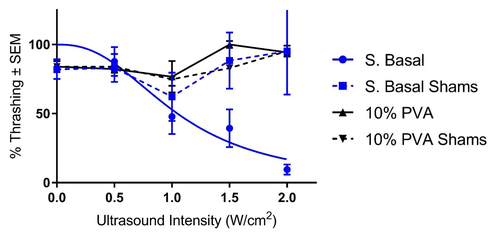
Insonated adults were transferred to NGM-OP50 plates. The next day, they were scored as **alive, dead, or missing**.

Worm survival decreased as the intensity of ultrasound was increased. At the high end of the range, only about 75% of insonated worms survived until the following day.

Determining the Mechanism(s) of Damage

We repeated these assays in polyvinyl alcohol (PVA), a viscous solution that is known to minimize cavitation[12]. **Insonating in PVA prevented the effects**, suggesting that **cavitation** was responsible.

Mutants assayed in S. Basal gave results similar to those of wildtype, suggesting that **release of reactive oxygen species (ROS)** and **apoptosis** were **not the main mechanisms** of damage or repair.



Conclusions

- After exposure to therapeutic ultrasound, worms exhibited dose-dependent reductions in **movement, fecundity, and survival.**
- Different bioeffects had **unique thresholds**.
- The main mechanism of damage appeared to be **cavitation** because effects were prevented by exposing worms to ultrasound in PVA.
- Mutants gave results similar to those of wildtype, suggesting that **cavitation masked ROS release** and that **apoptosis was insufficient** to overcome the tissue damage.

References

1. Torloni MR, Vedmedovska N, Merialdi M, Betrán AP, Allen T, González R, Platt, LD. Safety of ultrasonography in pregnancy: WHO systematic review of the literature and meta-analysis. *Ultrasound Obstet Gynecol* 33, 599-608 (2009).
2. Pay TL, Andersen FA, Jessup GL. Survival of *Drosophila melanogaster* pupa exposed to ultrasound. *Radiat Res* 75, 236–241 (1978).
3. Pay TL, Hellman KB, Sternthal PM. Effects of ultrasound on the longevity and reproductivity capacity of the female fruit fly *Drosophila melanogaster. Ultrasound Med Biol* 8, 549–552 (1982).
4. Rao S, Ovchinnikov N, McRae A. Gestational stage sensitivity to ultrasound effect on postnatal growth and development of mice. *Birth Defects Res. A Clin Mol Teratol* 76, 602–608 (2006).
5. Suresh R, Ramesh, Rao T, Davis EM, Ovchinnikov N, Mc Rae A. Effect of diagnostic ultrasound during the fetal period on learning and memory in mice. *Ann Anat* 190, 37–45 (2008).
6. Schneider-Kolsky ME *et al*. Ultrasound exposure of the foetal chick brain: effects on learning and memory. *Int J Dev Neurosci* 27, 677–683 (2009).
7. Xu S, Qiao Y, Liu X, Church CC, Wan M. Fundamentals of Cavitation. In: *Cavitation in Biomedicine: Principles and Techniques.* Wan M, Feng Y, terHarr G, ed. pp. 1-46. New York: Springer (2015).
8. Pizzarello DJ, Vivino A, Madden B, Wolsky A, Keegan AF, Becker M. Effects of pulsed, low-power ultrasound on growing tissues: I. Developing mammalian and insect tissue. *Experimental Cell Biol* 46, 179–191 (1978).
9. Child SZ, Carstensen EL, Davis HT. Test for "miniature flies" following exposure of Drosophila melanogaster larvae to diagnostic levels of ultrasound. *Experimental Cell Biol* 48, 461–466 (1980).
10. Child SZ, Carstensen EL, Lam SK. Effects of ultrasound on *Drosophila*: III. Exposure of larvae to low-temporal-average-intensity pulsed irradiation. *Ultrasound Med Biol* 7, 167–173 (1981).
11. Coakley WT, Hampton D, Dunn F. Quantitative relationships between ultrasonic cavitation and effects upon amoebae at 1 MHz. *J Acoust Soc Am* 50, 1546-1553 (1971).
12. Schelling G, Delius M, Gschwender AM, Grafe P, Gambihler S. Extracorporeal shock waves stimulate frog sciatic nerves indirectly via a cavitation-mediated mechanism. *Biophys J* 66, 133-140 (1994).
13. Frazier HN, Roth MB. Adaptive sugar provisioning controls survival of *C. elegans* embryos in adverse environments. *Current Biology* 19, 859-863 (2009).
14. O'Brien WD. Ultrasound-biophysics mechanisms. *Prog Biophys Mol Biol* 93, 212-255 (2007).
15. Dalecki D. Mechanical bioeffects of ultrasound. *Annu Rev Biomed Eng* 6, 229–48 (2004).
16. American Institute of Ultrasound in Medicine. How to interpret the ultrasound output display standard for higher acoustic output diagnostic ultrasound devices: version 2 [technical bulletin]. *J Ultrasound Med* 23, 723–726 (2004).
17. U.S. Food and Drug Administration. Avoid fetal 'keepsake' images, heartbeat monitors. *FDA Consumer Health Information* (2015).
18. Ding X, Lin SC, Kiraly B, Yue H, Li S, Chiang IK, Shi J, Benkovic SJ, Huang TJ. On-chip manipulation of single particles, cells, and organisms using surface acoustic waves. *PNAS* 109, 11105-9 (2012).
19. Isben S, Tong A, Schutt C, Esener S, Chalasani SH. Sonogenetics is a non-invasive approach to activating neurons in *Caenorhabditis elegans. Nat Commun* 6, 8264 (2015).
20. Hunter T, Bannister WH, Hunter GJ. Cloning, expression, and characterization of two manganese superoxide dismutases from *Caenorhabditis elegans*. *J Biol Chem* 272, 28652-9 (1997).
21. Burgess J, Hihi AK, Benard CY, Branicky RS, Hekimi S. Molecular mechanism of maternal rescue in the *clk-1* mutants of *Caenorhabditis elegans*. *J Biol Chem, 278*, 49555-62 (2003).
22. Ellis HM, Horvitz HR. Genetic control of programmed cell death in the nematode *C. elegans*. *Cell* 44, 817-829 (1986).