

THE BIOPHYSICAL EFFECTS OF ULTRASOUND

A Review of the Current Literature

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ULTRASOUND: AN OVERVIEW OF THE LITERATURE

Ultrasound (US) has been used for over 60 years in the therapeutic management of pain, musculoskeletal injuries. While it has been around for several decades, evidence of its favorable biophysical effects has only recently begun to accrue. (Barnett et al., 1994b)

In so far as the balance of evidence of US's clinical effects have derived from in vitro (outside the living organism) and animal studies, evidence for its beneficial therapeutic influence is rapidly accumulating. Caution must be applied, however, as the in vitro studies may not account for homeostatic (eg. thermoregulatory) mechanisms intrinsic to the living organism¹; animal studies may provide more insight into these.

Ultrasound is defined as sound wave having a frequency greater than 20 kHz (acoustic = 20-20,000 Hz). Two primary forms of ultrasound include diagnostic and therapeutic. Diagnostic ultrasound is used for medical imaging while its therapeutic counterpart is used in the treatment of various physical

¹ same intensities used in lysis studies do not produce adverse signs/effects in vivo

ailments; the combined. Diagnostic involves the emission of pulsed waveforms of less than 1 W/cm^2 intensity while therapeutic US typically employs incident waves of either 1 or 3 MHz, transmitted as either pulsed or continuous waveforms depending on the desired physiological effect(s). Although there are suggested demarcating points for thermal (continuous) and non-thermal (pulsed) ultrasound, the transition from thermal to non-thermal effects is graduated and ill-defined.

1. PHYSIOLOGICAL EFFECTS OF ULTRASOUND

1.1 Non-Thermal Bioeffects of Ultrasound

The primary and more important effects of US are due to non-thermal mechanisms mediated by a process called cavitation. Cavitation essentially describes the biophysical interaction of gaseous inclusions (bubbles) within tissues when exposed to an incident waveform (eg. ultrasound). Typically, bubbles will expand and contract as the peak positive and negative pressures propagate through the tissue and it is this oscillatory behaviour of the bubble that induces local biophysical effects.

Two forms of cavitation exist: stable (non-inertial) which is described as a phasic oscillation of the bubble within the ultrasound field; it has beneficial influence(s) on local soft tissues. Unstable (inertial) cavitation is described as a rapid collapse of the bubble which produces very high local temperatures and/or pressures resulting in the production of toxic 'free radicals' and eventual tissue damage. Stable cavitation is described as the phasic oscillation of gaseous inclusions, resulting in a mechanical vibration within the tissue in the absence of deleterious free radical formation. This vibration creates eddies of current flow around the oscillating bubble. Cavitation is rare in vivo owing to the lack of naturally occurring gas inclusions in living biological tissues (Dalecki, 2004). Baker (2001) suggests that it is difficult to demonstrate cavitation in VIVO at the intensities used for therapeutic US and, according to Health Canada "...there is no demonstrated risk of clinically significant damage in humans from mechanical effects of ultrasound exposure during a diagnostic examination".

The use of gas based contrast agents for imaging studies increases the potential for cavitation (Dyson, 1982). Having said that, under severe acoustic shock (high intensity exposure), mammalian cells have demonstrated cavitation. Reversible pulmonary extravasation (pulmonary capillary bleeding) has been has

been demonstrated, however, in mammalian lung due to unstable cavitation occurring at low level exposures (Child et al., 1990).

1.1.1. **In vitro Physiological Effects of Non-Thermal Ultrasound**

The in vitro bioeffects of ultrasound include:

-earlier resolution of inflammation, heightened fibroblast recruitment (Young and Dyson, 1990b).

-accelerated fibrinolysis (Harpaz, 2000;Francis et al., 1992).

-stimulation of fibroblast activity, increased protein synthesis, increased blood flow, tissue regeneration, bone healing, accelerated angiogenesis (Young and Dyson, 1990a;Young and Dyson, 1990c).

-increased matrix synthesis (Webster et al., 1980).

-increased collagen fibril density (Friedar, 1988)

-increased tissue tensile strength (Byl et al., 1992;Byl et al., 1993;Pocock et al., 2000).

1.2 Thermal Bioeffects of Ultrasound

US induced temperature rise varies with tissue properties (absorption coefficient, density, perfusion, pulse duration, PRF (pulse repetition frequency) and beam/scanning configuration. The unique property of US induced temperature rise is that it is focal, which may not trigger systemic heat-dissipating thermoregulatory mechanisms; febrile patients may also have difficulty dissipating heat.

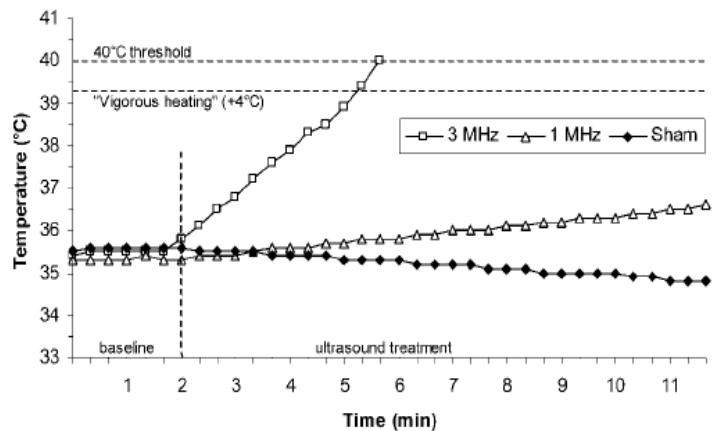
The factors that affect tissue temperature rise include the US field parameters, the involved tissue characteristics, thermal conductivity of tissue and blood perfusion of tissue. Temperature rises are steeper in tissues that are poorly vascularized (tendon, fat) and in tissues that conduct heat (bone). Tissues adjacent to bone are particularly susceptible to heat increase via conduction. Biological tissue absorption is directly related to the protein content (collagen has particularly high absorption). Generally, absorption is greatest in mineralized bone followed by skin/tendon, spinal cord and finally brain/liver/kidney.

The primary US field parameters to consider for thermal considerations include the wave form (pulsed, continuous), average and spatial intensity

(W/cm²) (Beam Non-Uniformity Ratio-BNR), time of exposure, duty cycle, frequency. Generally, pulsed waves produce less heat than continuous while intensity parameters determine the total energy transferred to the tissue.

Blood perfusion is poor in fatty tissue, tendon, sclera, periosteum and bone, making them more susceptible to heat-related effects. Non perfused tissues include cornea, lens and vitreous body of eye. Particular attention should be paid to lens of eye which can be heated due to high collagen concentration but cannot cool due to lack of adequate perfusion. The lens' protein content increases as we age and so does the absorption coefficient. Cataracts are the end result of excess US exposure to the lens.

Draper et al demonstrated that 1 MHz continuous US for 10 min 1.5 W/cm² [20 cm trans head/80 cm² surface area (4:1 ratio)] produced a 5 degree increase in the gastrocnemius muscle over 10 minutes at a depth of 3 cm without surface skin



Tissue temperature at 2.5 cm with 1.5-W/cm² ultrasound treatments.

heating. Horder (Horder et al., 1998c;Horder et al., 1998b;Horder et al., 1998a) found a mean peak temp increase of 4.3 deg C at the inner aspect of the skull parietal bone of late gestation guinea pig fetuses with an exposure of 2.8 W/cm²/SPTA for 120 sec. In the mouse skull, Carstensen (Carstensen et al., 1990) recorded temp elevations >5 deg C after 90 sec in anaesthetized mice exposed to continuous and/or pulsed US at 1.5 W/cm².

1.2.1 Positive Physiological Effects of Ultrasound

- a. Ultrasound induced heating has the unique property of penetrating up to 8 cm (1 MHz) to induce *focal* heating; surface application of heat, in contrast, is insulated by the fatty subcutaneous layers and severely attenuated.

- b. Ultrasound heating increases tissue extensibility (collagen fibers) in normal knees (Ellis, 1969) and scar tissue (Noyes, 1974).

- c. **Metabolic Effects:** The biochemistry of the cell is very temperature sensitive and it is difficult to imagine that US-induced heating does not play some effect in this, in spite of the paucity of data. It has been suggested that a 1 deg C increase in baseline temperature accelerates the metabolic rate in tissue and an increase of

2°C to 3°C reduces muscle spasm, joint stiffness, pain, and chronic inflammation and increases blood flow (vasodilation).

d. **Bulk streaming** is defined as liquid that flows within and parallel to US waves. This phenomenon does occur in vivo with tissue temperature elevations between 40-45 degrees for 5 min (Prentice, 1994) and promotes healing by bringing needed oxygen and nutrients to the tissues while removing waste from the injured site.

2. CURRENT THERAPEUTIC APPLICATIONS OF ULTRASOUND

The spectrum of use for ultrasound continues to expand. It has been used for over 3 decades in non-invasive diagnostic imaging of the body and has been/is being used for the following:

- a. **Lithotripsy** (breaking of kidney/gall stones by means of high amplitude sound waves) is routinely used to treat kidney stones.

- b. **High-intensity focused ultrasound (HIFU)** ($1\text{kW}/\text{cm}^2$) is being employed in the treatment of tumours by inducing mechanical/thermal damage to basement

membranes of capillaries (Moussatov et al., 1998). *Therapeutic* intensities of US are unlikely to cause basement membrane damage, *in vivo*.

c. **Nerve regeneration.** It has been demonstrated to facilitate post-traumatic peripheral nerve regeneration. (Paik et al., 2002; Lazar et al., 2001; Mourad et al., 2001; Crisci and Ferreira, 2002)

d. **Thrombolysis** ('clot buster'): A combination of pulsed US and thrombolytic drugs can accelerate the rate of clot dissolution *in vitro/vivo*; low frequency US much better for this purpose.

e. **Accelerated Fracture Healing:** Animal models demonstrate low intensity, pulsed US can be used to accelerate (by 30-38%) the rate of bone fracture healing non-invasively (Dalecki, 2004; Warden, 2003; Warden et al., 2001; Warden et al., 2000).

f. **Angiogenesis/arteriogenesis:** Hogan, Young and Dyson suggest vascular repair-inducing mechanical damage to basement membranes as a possible therapeutic avenue.

g. **Increased collagen repair:** Pulsed ultrasound treatment was found to result in better organization and aggregation of the collagen bundles in the repairing tissue than was the continuous wave mode of ultrasound treatment (da Cunha et al., 2001). Demir (Demir et al., 2004) also concluded that ultrasound increased the rate of tissue/tendon healing *biochemically* and *biomechanically* over a control group.

h. **Trigger Point**

Deactivation/Pain

Modulation: Our own

study demonstrated a 47%

decrease in myofascial

trigger point sensitivity with

one 5 minute ultrasound

session [1 W/cm² at 50% duty cycle for 5 min].

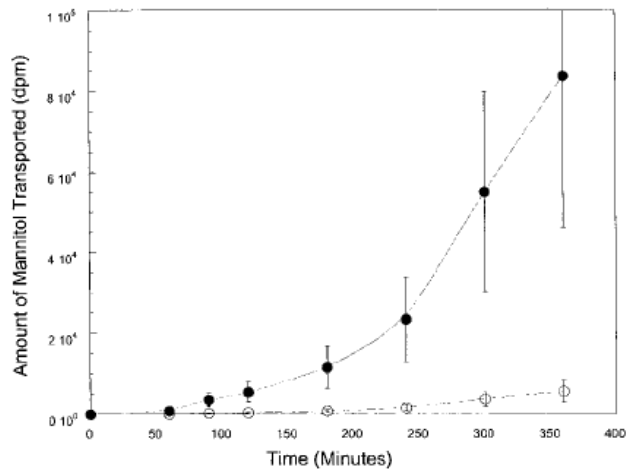


Figure 1. Transdermal delivery of mannitol through rat skin. Closed circles show transport across ultrasound-pretreated skin. Open circles show transport through untreated skin. The amount of drug transported is measured in units of dpm (disintegrations per minute).

i. **Phonophoresis:** Ultrasound can be used to drive medications (analgesics, anti-inflammatories) into tissue and enhance the site specific delivery of drugs. Dalecki (Dalecki, 2004) reports that pulsed US of 20-100kHz has been shown to significantly increase transdermal delivery of topical anaesthetics, insulin and

other high molecular weight proteins. Mitragotri (Mitragotri, 2005;Mitragotri and Kost, 2004;Mitragotri and Kost, 2001;Mitragotri and Kost, 2000;Mitragotri et al., 1996;Mitragotri et al., 1995) also confirmed that ultrasound at a frequency of 20 kHz significantly enhanced transdermal transport of drugs.

The specifications below represent optimal specifications for phonophoresis:

3 MHz, 20% duty cycle

0.5-0.75 W/cm²

5-10 min

2-3 x ERA

3. ADVERSE EFFECTS OF ULTRASOUND

The most damaging potential consequence of mechanical ultrasound is extravasation (capillary bleeding) in the lung and intestine due to the effects of cavitation, although Meltzer (Meltzer et al., 1998) was not able to corroborate this intraoperatively with transesophageal echocardiography. Valid animal studies, however, confirm hemorrhage with pulsed, 2 MHz US exposure for 3 minutes and similar results have been seen in animals at diagnostically relevant exposures, both in the lung and intestine (Dalecki et al., 1995). Tarantal (Tarantal

and Canfield, 1994) describes the observed hemorrhage as 'mild' and reversible, with no structural damage to the alveolar architecture. According to Health Canada *"It is unlikely that there would be any significant intestinal hemorrhaging, even at the highest MI values available."* Certain pathological conditions (decreased peristalsis) may increase the likelihood of gaseous inclusions and cavitation-related effects.

- a. The sensory organs (eye, ear, nervous tissue) are particularly sensitive to US as they are often situated close to bone and are vulnerable to conductive heat transfer. The neuroepithelium of the inner ear is readily destroyed by therapeutic doses of continuous (thermal) wave US (Barnett, 1980b; Barnett, 1980a). Many eye structures (lens, cornea) are rich in collagen (high absorption) yet avascular (poor perfusion) leaving them vulnerable to heat-induced damage.
- b. Thrombogenesis: lesions of the intima (inner lining of the blood vessels) may facilitate in thrombosis formation, especially in prone individuals (prolonged immobility, cancer, pregnancy, birth control).
- c. In VITRO studies have demonstrated increased cell lysis (death) at 1 MHz continuous US and increased damage to fibroblasts at both 1 and 3 MHz. These

observations were due to the effects of cavitation which is not a factor in VIVO (WFUMB News, 1997).

d. Cardiac premature contractions have been noted in frogs with 1.2 MHz exposure to a single 5 msec pulse (MacRobbie et al., 1997) with normal recovery.

A single pulse of high amplitude US can also affect transient contractility in animals (Dalecki et al., 1993b;Dalecki et al., 1993a;Dalecki et al., 1997).

e. Alterations in hematopoiesis (blood cell development) have been reported in monkeys after multiple exposures to US at diagnostic levels (Tarantal and Hendrickx, 1989;Tarantal et al., 1993).

f. While studies have been primarily directed at the gross effects on the cell, sub-cellular (cell membrane, signal transduction) effects are equally important yet less understood.

3.1 Ultrasound and the Developing Embryo

Potentially harmful effects of US are magnified in the developing embryo/fetus because of the rapid cell growth and proliferation. Focal lesions of

the parenchymatic organs (kidney, liver, thyroid), on the other hand, can be readily compensated for.

The developing nervous system is acutely susceptible to heat, physical or chemical damage as it proliferates. Hyperthermia has been shown to be teratogenic (fetus damaging) in many animal species, including human. While the fetus has a high absorption coefficient, it cannot respond well to heat induced irritation owing to their immature thermoregulatory mechanisms.

3.2 Ultrasound and Bone Marrow

Bone Marrow has been used as a model for rapidly proliferating cells and the fetal cerebral cortex since it's surrounded by bone.

Mild heat shock can elicit functional cellular changes that do not affect the integrity of the cellular structure.

-Barnett (1991): abnormal cell nuclei (in neutrophils) produced in guinea pigs with a sustained increase (6 min) of only 2.5 deg C.

-Roberts and Sandberg (Roberts, Jr. and Sandberg, 1979) demonstrated physiological change in human lymphocytes (enhanced response to migration factors) with a temp elevation of only 1.5 deg C.

Significant reduction in the production of neutrophils and monocytes following multiple exposures to diagnostic US in

monkey fetuses in utero has also been

demonstrated (Tarantal and

Hendrickx, 1987; Tarantal and

Canfield, 1994). Significant (but

transient) alterations in WBC were

also reported by the same research

group, both pre/post natally after

frequent exposure to US throughout gestation (Tarantal et al., 1995). These

studies' effects are due to primarily US-induced heating effects, not cavitation.

Table 1. Summary of studies of genetic effects of medical ultrasound.

Test system	Effect evaluated	Result
Bacteria	Genetic metabolic changes	Neg.
Yeast	Genetic metabolic changes	Neg.
Plants	Chromosomal aberrations	Neg./pos.
Insects [†]	Malformations	Neg./pos.
	Chromosomal aberrations	Neg./pos.
Mammalian cells <i>in vitro</i> [‡]	Chromosomal aberrations	Neg.
	SCE*	Neg./pos.
Mammalian cells <i>in vivo</i> [§]	Chromosomes	Neg.
	SCE	Neg./pos.
Laboratory mammals [¶]	Malformations	Neg.
	Fetal resorptions	Neg.
	Chromosome aberrations	Neg.
Human epidemiology	Leukaemias	Neg.
	Childhood cancers	Neg.
	Malformations, development	Neg.

[†] Wasps, and eggs, larvae and pupae of *Drosophila melanogaster*; [‡]lymphocytes, fibroblasts, CHO and HELA cells; *some experiments gave minor, but statistically significant, increases, which may be insufficient to produce a biologically significant effect; however, the biological relevance of SCE is obscure; [§]Lymphocytes, bone marrow cells, amniocytes and fetal mouse tissue; [¶]mouse, rat and guinea pig.

3.3 Animal Fetal Response to Pulsed Ultrasound

Animal studies demonstrated non specific stress response to pulsed US.

Rat embryo cultures have shown a stress response evoked by elevated

temperature (production of heat shock proteins, retarded embryonic development) of only 3.5 deg C (Edwards et al., 1997;Walsh et al., 1997;Walsh et al., 1987) or of only 1.5 deg C when insonated when pulsed US (1.2 W/cm² for 15 min) was applied together with the elevated temperature (Angles et al., 1990;Barnett et al., 1990). Heat shock proteins (72/73 dKa) were produced in living rat brain with temperature increases of only 2 deg C (from microwave heating). The oncogene, c-myc, was also activated under these conditions.

3.4 Mutagenesis

"There is at present no indication that medical ultrasound is capable of inducing mutations in mammalian tissue in vivo." (Barnett et al., 1983;Barnett et al., 1982). Dividing cells are more susceptible than non-dividing cells to the effects of mutagenic agents, therefore, mutagenic effects of US (or any other agent) may be more relevant in children and young people than older people. Although repeated chromosome aberrations (Barnett, 1997) and point mutations have been demonstrated in plants and insects (fruit fly), the table to the right illustrates little correlation between ultrasound and possible genetic alterations:

4. CONCLUSION

Ultrasound can interact with tissues to produce a variety of bioeffects through thermal and non-thermal effects. It has been employed for over three decades with very little documented evidence of adverse effect (Barnett et al., 1994a; Barnett et al., 1994b) and has a long-standing record of safety and efficacy in diagnostic imaging" (Dalecki, 2004). While more studies on the bioeffects of ultrasound are needed to better understand its impact on the body, its use as a therapeutic agent is becoming more widespread in rehabilitation clinics worldwide. Given the explosion of new information on the beneficial effects of US on the body, we can anticipate new and exciting advances in the field of ultrasound biotechnology

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