Editorial

A mechanism for ultrasound/light-induced biostimulation

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Low intensity ultrasound: non-pharmacological removal of *extracellular* Aβ

Alzheimer's disease was first identified more than 100 years ago, but 70 years passed before it was recognized as the most common cause of dementia and a major killer (1,2). The disease is primarily characterized by a massive decline in memory and cognitive skills, which is caused by neuronal dysfunction and damage, in particular in parts of the brain involved in cognitive function. Although research focusing on the mechanism of Alzheimer's has progressed a lot, much is yet to be done to prevent, delay or stop the disease. Expectations to ameliorate the disease are mostly fuelled by the pharmaceutical side. Virtually all of the 1,000,000 Google hits found for the key words breakthrough and Alzheimer's are related to some new drug. In view of this overwhelming trend, nobody expected the possibility of a potential solution from the physical side. Therefore, the recently reported substantial reduction of Aβ42 plaques in the brain of mice by the synergistic interplay of transcranially applied ultrasound waves and biologically inert micron sized microbubbles has a strong momentum of surprise (3). Leinenga and Götz report on two apparently (?) independent phenomena: the transient opening of the blood brain barrier (BBB) by ultrasound induced modulation (expansion and contraction) of the microbubbles (acoustic cavitation), and importantly, a concomitant reduction of the Aβ42 plaques in the brain of the test animals. These results were achieved in two subsequent steps: intravenous injection of microbubbles, and transcranial application of low levels of focused ultrasound waves with a pulse repetition frequency of 10 Hz. The duration of the treatment was 6 weeks—a promising scenario. Reportedly, the clearance of the Aβ42 deposits involved phagocytosis by microglia with uptake into lysosomes.

Notably, Leinenga and Götz were not the first to report on an animal model in which amyloid plaques were successfully removed by the physical method. In December 2014 Burgess *et al.* already reported that ultrasound waves in combination with microbubbles were instrumental in reducing amyloid plaques in mice (4). This independent corroboration is indeed encouraging, as pointed out by Foley *et al.* in a recent e-letter (5): "*With the paper by Leinenga and Götz, there is now evidence from two separate laboratories using two different AD mouse models to demonstrate that FUS and microbubbles alone can open the BBB and reduce plaque burden, with no unwanted damage to brain tissue.*" Nevertheless, reason and experience teach us to be prudent when it comes to the extrapolation of results obtained by a physical method in an animal model to humans. Several questions can be asked: will it be possible to adjust the ultrasound parameters so that the waves pass the skull of humans at intensities and doses which are sufficient to deliver the desired effect in the cortical region of the brain without producing external/internal thermal damage? In a paper exploring the utilization of ultrasound waves for drug delivery in a mouse model Choi *et al.* recognized the fundamental character of the problem (6): "*Focused ultrasound activation of systemically administered microbubbles is a noninvasive and localized drug delivery method that can increase vascular permeability to large molecular agents. Yet the range of acoustic parameters responsible for drug delivery remains unknown, and, thus, enhancing the delivery characteristics without compromising safety has proven to be difficult.*" Choi *et al.* proposed that the safety problem can be simply solved by the choice of proper sonication parameters: "*Our results have broadened the design space of parameters toward a wider safety window that may also increase vascular permeability.*"

As far as safety considerations are concerned three

critical aspects are missing in the literature: in order to satisfactorily predict or estimate possible damages caused by the ultrasound, by the microbubbles or by a combination of the two, we need to understand the precise interaction mechanism between each individual component and relevant cells. This requirement demands for suitable *in vitro* experiments. This standpoint receives support from the results of recent experiments which showed that low intensity pulsed ultrasound induced apoptosis in human hepatocellular carcinoma cells *in vitro* (7). The second critical aspect concerns the question: is a short lived mouse neuron a good model to ascertain (exclude) possible long term damages caused by the treatment in human neurons? The third critical aspect concerns the question whether it is permissive to apply a "safety window" established in a mouse model to humans.

Low intensity laser light: non-pharmacological removal of *intracellular* Aβ

With the aforementioned justification for suitable *in vitro* experiments we want to postpone a comprehensive discussion regarding details of the interaction mechanism. Instead we shall go ahead and focus on possibilities which may allow us to improve the efficacy of a potential therapy based on the use of ultrasound. Whereas low intensity ultrasound waves recommend themselves for non-pharmacological removal of extracellular Aβ deposits, low intensity laser light (wavelength 670 nm) could be the ideal complement for non-pharmacological removal of intracellular Aβ deposits, as recently demonstrated in an *in vitro* model (8). The relevance of intracellular Aβ deposits in Alzheimer's disease has been emphasized earlier (9). Apparently, 670 nm laser irradiated neuroblastoma cells reduced their previously internalized Aβ loads via autophagocytosis, an ATP-fuelled process. A further synergistic complement to the ultrasound based method is the perspective to open the BBB by low intensity laser light, i.e., by the same light which is used to induce autophagocytosis (10). In contrast to the ultrasound based method, which requires for an opening of the BBB the injection of microbubbles, 670 nm laser light is envisaged to do the job alone. Thus, the temporal and spatial coordination of ultrasound, 670 nm laser light and suitable drugs appears exciting.

There is a here striking historical analogy to the situation which was previously common to the field of low level laser therapy (LLLT). While the clinical use of low intensity lasers was already established in the seventies, for many years the intrinsic interaction mechanism by which the photons increased the proliferation of cells *in vitro* or accelerated the healing of complicated diabetic ulcers in patients (11), for example, was not understood. Clearly, as long as the molecular pathways underlying a therapeutic effect are not understood it is impossible to systematically apply its principle. Instead everything is reduced to a trial and error process. Even worse is the situation when it is attempted to explain a manifest therapeutic effect using an incorrect model. Instead of enhancing the predictive capability of the model it will restrict it. Actually there is a manifest asymmetry between the clinically relevant laboratory results of the ultrasound based method and the understanding of the underlying interaction mechanisms. It is instructive to compare this situation to the one which previously prevailed in the field of LLLT: initially, there was a manifest gap between increasing clinical acceptance of LLLT, on the one hand, and deficit in the understanding of the underlying interaction mechanism, on the other hand. Eventually this asymmetry mobilized enormous research efforts. The spectrum of potential applications of LLLT includes, but is not limited to, accelerated healing of wounds, treatment of tumors, Alzheimer's disease, Parkinson's disease, and perhaps even anti-aging, as recently reported (12). Initially, the interaction mechanism by which low intensity laser light presented beneficial effects in cells as different as muscle cells, brain cells and skin cells was not understood. In 1984 Passarella *et al*. reported that irradiation with low intensity laser light increased ATP production in cells (13). The authors suggested that ATP is produced by a laser-induced proton-motive force. Unfortunately, the authors did not pursue the hypothesis because at that time it was practically impossible to prove it. Soon the proton-motive force hypothesis was forgotten and replaced by the assumption that the laser energy is converted into ATP by the enzyme cytochrome c oxidase (14). In our opinion, the "cytochrome enzyme mechanism" is not capable to explain the enhancement of the action of the ATP synthase. First, red or near-infrared (NIR) light is practically not absorbed by the chromophore of cytochrome c oxidase (it is a porphyrin ring with an absorption maximum at 550 nm). Second, the ATP synthase does not need light, it works using a pH gradient, as was first postulated by Mitchell (15) (that time, experts laughed about Mitchell's hypothesis). There exist experiments showing that the concentration of cytochrome c oxidase increases slightly upon illumination with NIR light. However, these results are not understood and they do not prove any connection between cytochrome c oxidase and

ATP synthase.

Two methods—one root cause mechanism?

Recently, we communicated the results of laboratory experiments showing that the viscous friction mediated by nanoscopic interfacial water layers confined between proximal surfaces is massively reduced by irradiation with low intensity 670 nm laser light—virtually the same light which is clinically used in LLLT (16) and which was previously shown to upregulate ATP levels in cells *in vitro* (8). In a first attempt to find an experiment-based and logical connection between the light treatment and the enhancement of ATP production, in particular in the case of cells under stress, we proposed the idea that irradiation of cells with low intensity 670 nm laser light reduces the viscosity of the nanoscopic interfacial water layers within and around the ATP synthase, thereby facilitating its normal (or improved) rotation. Normally, the mitochondrial rotary motor is turning 9,000 times per minute to fuel the cell with ATP. The new mechanism based on laser-induced interfacial lubrication was predicted to counteract effects of oxidative stress, thought to reduce the performance of the mitochondrial rotary motor via an increase in interfacial viscosity (16), and provides an explanation of how the light effect might work in cells exposed to stress—without intervening in the mitochondrial electron transport chain.

Every time when there is a fundamental discovery people try to apply it to as many unsolved problems as possible. Thus, it is tempting to put forward the hypothesis that the phagocytosis of Aβ by microglia which was shown to remove extracellular Aβ deposits from the brain of the mouse was fuelled by low intensity ultrasound waves, similarly to the mechanism proposed by us for low intensity laser light. Our hypothesis receives support from the experimental side: low intensity ultrasound was found to upregulate ATP levels in cells *in vitro* (17). It is perhaps not simply a coincidence that the therapeutic spectrum of low intensity ultrasound resembles to a large extent to that of LLLT. Ultrasonic stimulation was recently recommended to accelerate the healing of complicated diabetic ulcers (18). These results could motivate the experimental side to further explore whether it is possible to reduce the viscosity of nanoscopic interfacial water layers with low intensity ultrasound waves—in analogy to our previous work (16). In addition, the similarities between the biological effects of low intensity ultrasound and low intensity laser irradiation are sufficient motivation for the design of *in vitro* experiments which allow us to interrogate intracellular viscosities directly. Pioneer work in this direction was done by Sorscher at Lawrence Berkeley Lab in 1979 (19). If indeed our model is correct and possible doubts concerning long-term safety have been eliminated then therapies based on low intensity ultrasound and microbubbles (20) complemented by low intensity laser light (continuous wave or pulsed) (21) together with suitable pharmacological agents may become reality.

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Footnote

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