Fundamentals of the molecular-biological effect of extracorporeal shockwaves on the human organism

In vitro and in vivo examinations

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To date only the physical parameters associated with extracorporeal shockwave therapy (ESWT) have been exactly defined and made available, this by way of essentially empirical examinations of the clinical results of ESWT.

In contrast to the physical effects, the biological and / or molecular-biological changes induced by using ESWT are essentially still unknown and have been little investigated.

The molecular-biological effect of extracorporeal shockwaves is essentially mechanical stress which can bring about the liberation of certain of the body's own substances, these being capable of establishing communication between individual cells and, as well, enabling signal transmission within the cells. This signal transmission (or signal transduction) is of vital significance to maintaining the specialized functions of the signal cells. Signal transduction involves a directed flow of information; its signals can be seen as biological information units which provoke certain biochemical changes in the cells for which the signal is intended. In so doing the organism makes use of various signal transmission vectors, both intercellular and intracellular. Some cells form intercellular channels between themselves, the so-called "gap junction" or "cell-to-cell channels" through which small molecules can pass and trigger physiological processes in the partner cells. In addition, communication may transpire by way of receptors located on the cell walls or by liberating so-called neuroendocrine transmitters which can then cover greater distances on their way to the target. Ultimately all intercellular signal transductions will result in intracellular stimulation and activation of the target cells in order to induce functions specific to those cells.

Extracorporeal shockwave induction represents a complex, signal-emitting stress situation which manifests itself at various levels, those levels interacting one with another in numerous ways. Among the effects is the generation of free radicals which had in the past been associated essentially with the triggering of so-called oxidation stress. More recent investigations have revealed, however, that they play an important role as signal and modulator modules, particularly in the activation of cellular defenses against the effect which various stressors exert upon the cell itself.

The free radicals are understood to be fragments which possess one or more unpaired electrons; as a result of these electrons the radicals can exhibit pronounced chemical reactivity. The most important among them are the superoxide anion radical ($^{\cdot}O_2^-$), the hydroxyl radical ($^{\cdot}OH$) and the nitrogen monoxide radical ($^{\cdot}NO$).

Depending on the central atom in each case, these free radicals are divided by definition into reactive oxygen species (ROS) or reactive nitrogen species (RNS). These are referred to collectively as "reactive oxygen and nitrogen species" (RONS).

The changes in the cellular redox status initiated by RONS, by oxidation and by nitrosylation of other atoms and molecules, form the basis for their decisive role as signal and mediator molecules. Important is that any uncontrolled progression of such reactions can damage cells and even result in cell death.

The induction of the endogenic, antioxidant protective systems in response to mechanical stress (ESW) might be triggered by activation of the superoxide dismutase (SOD) located in the mitochondria and cytosol (although this has yet to be demonstrated), representing a primary defense against $O_2^{\cdot -}$, along with the selene-dependent glutathione peroxidase (GPx) and catalase, which inactivate H_2O_2.

In order to detect these free radicals, created in response to mechanical stress induced by the extracorporeal shockwaves, we made use of electron paramagnetic resonance

spectroscopy (EPR), with which we could unequivocally demonstrate the generation of free radicals due to the influence of ESW. Among the substances we found was nitrogen monoxide (or nitric oxide), the outstanding biological significance of which has in the meantime sparked great interest in scientific literature.

Seen chemically, ˙NO is a colorless gas of limited solubility in water which, in spite of its radical character, is relatively stable, exhibiting only moderate chemical reactivity. Biosynthesis from arginine has been explained, in just the same way as the ˙NO synthases, of which there are three isoforms (endothelial, neuronal and inducible NOS). The most important properties of NO are:

1. The activation of the guanylyl cyclase due to the reaction of ˙NO with the haemo iron of this enzyme, with subsequent vasodilation.
2. The neuroprotection of $˙NO^+$, observed under oxidative conditions, which is associated with the interaction of $˙NO^+$ with thiolate ions (cysteine residues) in the ion channel of an NMDA (N-methyl-D-aspartate) receptor. What results from this is the deactivation of neurotransmission of the excitatory (exciting) amino acids such as glutamate.
3. The foreign body defense found in phagocytosis caused by induction of the iNOS, associated with the liberation of larger quantities of ˙NO and simultaneous presence of active oxygen species (respiratory burst) (ROS).

Since the measurement of ˙NO using EPR spectroscopy is very complex and can at present be carried out only in vitro with the concomitant inaccuracies, we sought a different method which makes possible direct ˙NO detection in vivo. Here we made use of the so-called ˙NO analyzer made by the Thermo Instruments company, used originally in environmental protection work to measure nitrogen monoxide content in the air. This extremely sensitive process makes use of a reaction of ˙NO with ozone. This gives rise to energetically excited NO_2 molecules which emit radiation in a broad wavelength band of from about 600 to 1200 nm as they fall back to lower energy levels. This process is perceived spectroscopically as chemiluminescence, the intensity of which is virtually proportional to the ˙NO concentration.

Discrimination of the process is ensured by the fact that only gaseous substances which exhibit chemiluminescence due to reaction with ozone can be detected.

As has been discussed by Duchstein et al. it is essentially only an exogenic .NO synthesis which takes place in the skin as a result of external influences. The endogenous, NOS-catalyzed .NO synthesis from L-arginine can be realized due to external influences only in deeper tissue layers. One can use this factor for in vivo testing of the inducement of .NO by mechanical stress factors, e.g. by way of extracorporeal shockwaves. Using the .NO analyzer we measured the skin's .NO emissions first without shockwave exposure and then at various periods of time after exposure. The difference between the T-zero value and the value after exposure then provides information on the creation of endogenous .NO emitted through the skin. Testing carried out to date indicates increased .NO production for as long as 24 hours after exposure.

A further marker of the interaction of the organism with high-frequency, extracorporeal shockwaves is the detection of ultraweak photon emissions (UPE). Four living things are capable of emitting light. These photoreactions result as a rule from the chemical reaction among ATP – the cell's energy supplier, oxygen and luciferin and are visible with the naked eye. The quantum yield of such a reaction is extraordinarily high and can come to as much as 95%.

But reactions induced by reactive oxygen species and/or free radicals can also transpire under the participation of light. Most exogenic, oxidative reactions, however, produce heat in the main. Only a very small amount (less than 1%) of the enthalpy of reaction is liberated as light.

The emission of electromagnetic radiation by atoms or molecules in the UV, visible or IR spectrum following the transition of an electron from a higher energy level into an unoccupied lower energy level is referred to as luminescence. In chemiluminescence the luminescence is created directly by a chemical reaction; mechanical energy – extracorporeal shockwaves, for instance – results in so-called triboluminescence.

The ultraweak photon emission (UPE) describes luminescence phenomena of very low intensity in biological systems or specimens, without the use of light-intensifying systems such as luminol or lucigenin. In the literature this is described as low-level chemiluminescence, dark chemiluminescence, low-intensity chemiluminescence, delayed chemiluminescence or ultraweak luminescence.

UPE is subdivided into spontaneous photon emissions, i.e. a photon emission without external excitation, the observation of which requires extremely sensitive detectors, and induced photon emissions, i.e. photon emission

in response to external excitation. It is this latter form of UPE has been researched the most extensively since it is easier to detect than spontaneous photon emissions. Excitation can be effected by physical or chemical stressors. An example of such a physical stressor would be high-frequency, extracorporeal shockwaves; it was after their application that we measured the UPE level at the skin. The quantification of the UPE can be taken as a measure of the physical stress. Under this premise we examined two ESWT devices on the market in regard to the consistency of their effects. Here we determined that there was a rapid loss of efficacy in both devices. In one of the units only about 20% of the stated effectiveness could be used. After a brief period of this time the effectiveness of this device approached zero. The same was true for the demonstration of NO liberation. With the "aging of the electrodes" the detection of NO liberation deteriorated ever further, ultimately approaching zero.

As a further protective system, in addition to the enzymatic-antioxidative defense, the organism unfolds an additional mechanism to thwart the influence of RONS by forming so-called stress proteins. Among these are the so-called heat shock proteins (HSP) which can be formed, rapidly and in large quantities, as the cellular response to externally induced stress, whether it be physical or chemical in nature. They are found in almost all eukaryotic cells examined to date and are divided into groups according to their relative molecular masses, whereby the number contained in the abbreviation reflects the approximate molecular mass in kilodaltons. Considered to be cellular stress inducers are hyperthermia, ischemia, protein denaturation by various chemical or physical effects, and physical exertion. Thus it seemed obvious to examine whether it was possible to demonstrate the formation of heat shock proteins as a result of mechanical stress induced by way of extracorporeal shockwaves.

Our experiment thus comprises exposing the Achilles tendon – at the transition to the gastrocnemius – to extracorporeal shockwaves. Fine-needle biopsies were carried out in the treated area prior to exposure, 3 hours after exposure and 24 hours after exposure; heat shock proteins were detected using monoclonal HSP antibodies in the Western blot technique. There was found to be a significant expression of HSP 70 after three hours, which was distinctly strengthened after 24 hours. Similar results were found at the mRNA level.

Based on the examinations carried out to date, we may state in summary that the organism's self-protection mechanism is activated in response to mechanical stress induced by extracorporeal shockwaves and thus the regeneration of pathological processes, be they either chronic or acute in nature, can be stimulated and accelerated.

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