Extracorporeal Shock Waves: perspectives in malignant tumor treatment

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Abstract

Progress in basic research led to the design of new generations of anticancer drugs with some notable achievements. Over the years, more and more powerful drugs have been developed with the purpose of increasing the rate of response to therapy. As molecular power of chemotherapeutic agents increased, unfortunately also toxicity and undesired side-effects increased as well. The search for new therapeutic strategies to be used in the management of cancer is one of the more promising strategies to reduce chemotherapy toxicity. Extracorporeal Shock Waves (ESW), widely used for the treatment of urolithiasis, have been reported to cause modifications of cell growth both *in vitro* and *in vivo*. They exert an agonist cytotoxic effect with several chemotherapeutic agents like cisplatin, doxorubicin, bleomycin, paclitaxel and, moreover, as it has been reported that their main mechanism of action is an increase in cell membrane permeability, ESW are also used to deliver oligonucleotides and other small particles to cells. Recently, it has been found that certain dye compounds, in particular porphyrins, can achieve a cytopathogenic effect when the disease site is subjected to ultrasound irradiation. This technique is referred to as sonodynamic therapy. Based on the new knowledge about the interaction between ultrasound with bulk liquid, several studies have shown a synergic effect of ESW and porphyrins *in vitro*, thus opening a new perspective in the sonodynamic therapy, able to overcome some drawbacks encountered during conventional anticancer drug treatment. Finally, the current advances in bioengineering encouraged the application of nano-scale technologies to medicine. Nanobubbles, composed of an external shell and a gas core, can deliver chemotropic drugs and porfirins, to tumour target tissues in response to physical triggers, and ESW features make them an ideal alternative to ultrasound in combination with drug-loaded nanobubbles in delivery strategies.

Introduction

The mainstream of non-invasive therapies for treating solid tumors is by far chemotherapy**.** Taking into account that a crucial prerequisite for any cancer therapy is that the benefits of killing cancer cells outweigh deleterious side effects, it is admitted that any chemotherapy scheme is limited by both sub-optimal specificity for cancer cells and the probability to induce suppression of the host anti-cancer immunity. Over the years, more and more powerful drugs have been developed to increase the rate of response to therapy. As molecular power of chemotherapeutic agents increased, unfortunately also toxicity and undesired side-effects increased as well. Therefore, the search for new therapeutic programs to be used in the management of cancer, like innovative methods to deliver drugs to cancer cells, immunotherapy and gene therapy, in addition to the development of new pharmaceutical molecules, is one of the more promising strategies to reduce chemotherapy toxicity.

Extracorporeal Shock Waves

Extracorporeal Shock Waves (ESW) are high-energy acoustic waves produced by a generator through the conversion of electrical energy to mechanical energy. They propagate in water medium and are characterized by a definite shape with an initial positive, very rapid part of high amplitude, followed, after a few microseconds, by a sudden phase of mild negative pressure, before returning to the basic values. There are three main techniques through which ESW are generated. These are the electrohydraulic, electromagnetic, and piezoelectric principles, each of which represents a different technique of generating the shock wave (1,2).

There are two basic effects of ESW; the primary effect is the direct generation of mechanical forces that result in the maximal pulse energy concentrated at the point where treatment is to be provided; and the secondary effect is the indirect generation of mechanical forces by cavitation which may cause damage to the tissues (1-3).

Shockwaves have been used in medicine for many years, particularly in extracorporeal lithotripsy (ESWL), which uses focused shockwaves to treat non-invasively patients with stone diseases (mostly, urinary stones) (1,2). The excellent results achieved by ESWL stimulated research on the applications of focused shockwaves in other branches of medicine. Recent progress in antitumor target therapy and delivery systems triggered by physical forces reinforces the use of ESW as a new tool to be used in anticancer delivery strategies. The present review highlights the different anticancer strategies using ESW: the cytocidal effect of ESW alone or in combination with chemoterapic drugs; the sonodynamic therapy; the ESW-aided gene transfer; the nanotechnologies (Table 1).

Cytocidal effect of ESW alone or in combination with chemoterapic drugs

Cell membranes, which have a thickness of a few molecular layers, are subjected to extremely high pressure gradients at the transit of ESW. For this reason, in the late 80s - early 90s, some authors took into account to expose a spatially limited region of the body to a potentially destructive form of mechanical energy. They hypothesized to take advantage of the cytotoxic/cytocidal effect of ESW, until then exclusively used for the treatment of urolithiasis, which could be regarded as an important additional support in cancer treatment. Appropriate *in vitro* and *in vivo* studies showed that ESW could cause only temporary growth delay. Nevertheless, considerable morphological changes at the cellular level were observed, including effects on plasma membrane, mitochondria, cytoplasm and nucleus (4-6). These damaging effects could sensitize tumor cells to most cytotoxic agents. Further studies (7-8) suggested that cell membrane permeabilization is the most prominent alteration induced by essentially sublethal doses of High Energy Extracorporeal Shock Waves (HESW). With respect to the potential side effects of ESW treatments, permeabilizing ESW energies were observed to induce cell mortality in a dose-dependent manner (9). Thus, concurrent treatment regimens with ESW and hydrophilic drugs looked promising since ESW can regionally render tissue more susceptible to the drug with the prospect of reduced systemic toxicity. ESW were reported to cause modifications of

cell growth both *in vitro* and *in vivo* (5, 10). Zhou and Guo (11) observed, in nude rats, that high energy ESW treatment was able to delay tumor growth and reduce tumor size, without evidence of metastasis.

A substantial difficulty in comparing the results obtained by different Authors is due to the large heterogeneity of mechanical, biological and analytical variables in each experimental procedure. *In vitro* studies have shown that ESW treatment elicits immediate reduction of cell viability and ability to form colonies (5). Different sensitivities to ESW of different cell lines have been described by Brummer et al. (12). Moreover, the impact of ESW on cell survival varies not only with the cell type, but also between cell lines of the same type (13); it was hypothesized that cells in G_2-M phase cell cycle can be more easily damaged by ESW as compared to cells in G_0.

With regard to the mechanism by which ESW cause cellular damage, numerous hypotheses have been advanced. Based on the fact that cells did not show any damage when immobilized in agar, Brummer et al. (14) suggested that the collision between cells could play a role in changing their viability; it was subsequently proven that viability was not influenced by varying cell concentration (13). It was also suggested a possible role of free radicals that are generated by Shock Waves in inducing cellular damage. Currently, the best hypothesis on the mechanism of cell damage elicited by Shock Waves is that of cavitation and the generation of jet streams in the extracellular milieu. The cells that survive after ESW exposure are still able to form tumors when inoculated in animal models, but these tumors are smaller than in controls (ESW-untreated) since a significant percentage (40 to 60%) of surviving cells that have been inoculated showed sublethal induced damages (5).

Numerous studies have shown that the combined treatment of tumor cells in suspension with some anticancer agents and ESW elicits a significant enhancement of drug cytotoxic effect (15,16). It was noted that Shock Waves, when applied to cells *in vitro*, determine (even at low energy) a transient increase in cell membrane permeability by opening pores, allowing higher concentrations of drug enter into the cells (17,18). This effect is similar to that obtained through the technique of

electroporation, as direct access to the cytoplasm was also gained by high voltage electric pulses. Electroporation was shown to enhance the action of bleomycin and it had been clinically applied to subcutaneous tumor nodules (19). It has to be pointed out that a major problem of electrochemotherapy is that the electrodes have to be in close local contact over the whole tumor surface. This prohibits its use in internal organs. ESW can, in contrast, be directly administered to internal organs such as the liver or the gut, and no surgical intervention is necessary.

Cells of human estrogen-dependent breast cancer (MCF-7) were sensitive to combined treatment with ESW and paclitaxel, an antimicrotubule agent, active against a variety of solid tumors (9). The suppression of cell proliferation induced by Shock Waves has been related to an apoptotic mechanism (16). Apoptosis, i.e. programmed cell death, is a cellular self-destruction mechanism, which plays a key role in the surveillance against tumors (20). Induction of apoptosis occurs in response to a variety of stress signals (21) which may include Shock Waves (16).

Recent studies have shown the cytotoxic action enhanced by Shock Waves in combination with some anticancer drugs *in vitro*: cell lines of human osteosarcoma (22), human colorectal adenocarcinoma (23) and human anaplastic thyroid cancer (24) were subjected to combined treatment (ESW and anticancer drugs). Combined exposure to anticancer drugs and Shock Waves resulted in a significant enhancement of cytotoxicity and induction of apoptosis in cancer cells.

Sonodynamic/photodynamic properties of ESW

In the work by Catalano et al. (24) an innovative "sonodynamic/photodynamic" technique was adopted, based on ability of ESW to activate and render cytotoxic a photosensitizing substance: the natural porphyrin precursor 5-aminolevulinic acid (ALA), which is accumulated selectively by neoplastic cells.

In normal cells, protoporphyrin IX (PPIX), a substance with excellent photosensitizing properties, does not accumulate to a great extent because it is quickly transformed to heme by the action of ferrochelatase. In cancer cells, however, PPIX accumulates due to a defective heme biosynthesis, as

a consequence of abnormal levels of some enzymes involved in this pathway. Increased activity of porphobilinogen deaminase and/or decreased activity of ferrochelatase have been reported for a number of tumors. Exogenous application of ALA can lead to a pronounced accumulation of PPIX in tumor tissue and subsequent irradiation with light of wave lengths (corresponding to the PPIX absorption bands) can lead to specific destruction of tumor cells. Photodynamic therapy (PDT) has developed as an important new clinical cancer treatment modality in the past 25 years but the low penetration depth of light through the skin and tissues has limited PDT to the treatment of superficial, endoscopically reachable tumors. The main assumption about "sonodynamic" therapy is to generate ultrasound energy which produces sonoluminescence to excite the protoporphyrin derivative by energy transfer. HESW induce acoustic cavitation, which results in a concentration of energy sufficient to generate a sonoluminescence emission, able to cause electronic excitation of porphyrins and initiate a photochemical process resulting in the formation of the cytotoxic singlet oxygen (25). The mechanisms underlying this effect were explained on the basis of double basic effect elicited by HESW treatment: the direct generation of mechanical forces (non-inertial cavitation) and the indirect generation of mechanical forces by cavitation (inertial cavitation). Non-inertial cavitation bubbles oscillate and cause streaming of the surrounding liquid and mechanical stresses. Inertial cavitation is an extremely violent process of bubble activity that may generate highly reactive hydroxyl radical. The subsequent energy transfer to oxygen can generate the highly reactive singlet molecular oxygen (25). This combination between inertial and non-inertial cavitation, generated by a piezoelectric Shockwave device, was confirmed by Canaparo et al. (23), who observed that combined ALA-high energy ESW treatment produced significant inhibition of HT-29 (human colorectal carcinoma) cell growth. Non-inertial as well as inertial cavitation was seen to induce apoptosis as well as to inhibit cell growth by increasing the G_0/G_1 population through the intracellular activation of protoporphyrin IX.

"Sonodynamic therapy" is an analogous approach to PDT based on the synergistic effect of ultrasound and chemical compound referred to as "sonosensitizer", but the attractive feature of this modality for cancer treatment emerges from the ability to focus the ultrasound energy on malignancy sites deeply placed in tissues. The ESW source can be placed at direct contact with the body and the maximum energy flow given to the inner part of the tumor can be precisely controlled. Nonetheless, at transition sites between tissues with different acoustic impedance values, there may be focal mechanical destruction, probably through the induction of cavitation and shearing stress caused by the reflected waves (26).

This technique has proven to be effective *in vivo*, by inducing necrosis and apoptosis of breast cancer and colon cancer implanted in laboratory animals (27,28).

Most recently, the anticancer effect of Sonodynamic therapy (SDT) using ESW-activated protoporphyrin IX cytoxicyty on a syngeneic rat breast cancer model was confirmed by magnetic resonance imaging (MRI). The SDT-treated group showed a significant decrease in MRI tumor size measurements 72 hours after treatment with the PPIX precursor ALA and ESW (29).

Nano-scale technology and ESW

The current advances in material science and bioengineering encouraged the application of nano-scale technologies to medicine; nanocarriers (or nano-encapsulation systems) have been introduced as promising vehicles in drug delivery. In such systems, drugs and bio-active agents are wrapped in or adsorbed on the surface of nanoparticles in order to achieve safe and effective drug delivery and gene therapy (30). When compared to traditional delivery systems, nanocarriers used in chemotherapy have higher therapeutic efficiency at low dosage and can accumulate preferentially in the desired locations due to the defective vascular architecture of most solid tumors. This enables the nanocarriers circulating in the blood to be entrapped inside the leaky vessels of the tumor, thus slowly releasing their contents. In recent years, a wide range of nanoparticles (NPs) have been employed as drug-delivery carriers for cancer therapy. NPs can carry loaded drugs to the tumor site

through the blood stream taking advantage of the enhanced permeability and retention effect, due to the defective vascular architecture of the tumor tissue (31). Actually, growing attention in the field of nanomedicine has been given to micro- and nanobubbles (NBs). NBs, composed of an external shell and a gas core, can deliver diverse molecules, such as DNA and drugs, to target tissues in response to physical triggers, like ultrasound. Based on their features, ESW may be considered an ideal alternative to ultrasound in combination with drug-loaded NBs in delivery strategies. We recently demonstrated that combining new doxorubicin-loaded glycol chitosan NBs and ESW enhanced doxorubicin anti-tumor activity in anaplastic thyroid cancer cell lines, by increasing intracellular drug release (32).

Moreover, nanoparticles may enhance the sonodynamic therapy response by playing different roles: if properly engineered, the sonosensitizer agent loaded onto NPs, can pass more readily across the cell membrane, reaching its critical intracellular target. It has been demonstrated that sonosensitizers loaded onto NPs are more readily taken up by cells with respect to the free drug (41). Moreover, NPs are able not only to function as a sonosensitizer per se, but also as energy transducers (33). The synthetic water-soluble TPPS [meso-tetrakis (4-sulfonatophenyl) porphyrin] has been widely investigated as a photosensitizer because of its high tumor tissue affinity and retention rate, as well as a remarkable quantum yield of singlet oxygen formation in solution (34).

Quite recently, an innovative sonosensitizing system using ESW and a new formulation of nanoparticles [poly-methyl methacrylate core-shell nanoparticles (NPs)] loaded with TPPS has been described (35). The sonodynamic treatment with nanoparticles loaded with porphyrin derivative (TPPS-NPs) and ESW was investigated with regard to cytotoxic effect on the human neuroblastoma cell line, SH-SY5Y. Single cell treatments, such as exposure to ESW alone or TPPS alone, had no effect on SH-SY5Y cell proliferation. Indeed, the combined treatment with TPPS-NPs and ESW, showed a statistically significant decrease in SH-SY5Y cell proliferation. This new sonosensitizing system significantly decreased cancer cell growth after ESW exposure, even in a three-dimensional

model of neuroblastoma, suggesting its potential further application in sonodynamic anticancer therapy (35,36).

Cancer gene therapy and ESW-aided gene transfer

Cancer gene therapy has made important progresses. One established immunotherapy approach involves enhancement of the immune response against cancer through the augmentation of natural cytokines. These proteins can either be repeatedly injected or produced by plasmid DNA transfer and protein expression in cells of the tumor or host animal. Immunogenic therapy using pIL-12 has shown promise in some immunogenic mouse tumor models (37). However, systemic application is problematic, since effective IL-12 doses are toxic to the animal. Thus, the search for new delivery methods remains of great interest, particularly for established tumors. Physical methods for membrane permeation, along with their ability to promote cell transfection, may overcome the barriers of gene transfer using non-viral vectors. These methods include electroporation, direct injection, biolistic particle delivery, laser irradiation, magnetic nanoparticles and acoustic cavitation. Because of their adaptability to *in vivo* purposes (easy to use, minimal effects on normal physiology), electroporation and acoustic cavitation seem to be the most promising techniques for gene therapy applications. In particular, the utility of acoustic cavitation for cell permeation and transfection has been illustrated in different cell types, both *in vitro* and *in vivo* (38,39).

In the last decade some studies showed that Shock Waves can support transfection, i.e. the transfer of therapeutic genes to targets by temporarily increasing cell membrane permeability: DNA - or fragments, such as plasmids - can enter the cell (18,37,38,40). The combination of shock waves and DNA cationization for cell transfection was explored on an *in vitro* model of suspended cells and GFP (green fluorescent protein) reporter-containing plasmid DNA (41). In terms of percentages of transfected cells, the efficiencies found were comparable to those reported by other acoustic cavitation-based approaches (42).

ESW-aided gene transfer has been associated with many advantages in cancer gene therapy: 1) localization of DNA transfer to the tumor; 2) cavitation-induced cell killing resulting in tumor ablation, and, 3) the avoidance of antigenic responses associated with virus-based DNA delivery methods (43,44). Quite recently a synergistic approach has been described using ultrasound and nanoparticles to deliver plasmid DNA to cancer cells, achieving really high transfection efficiency and enhanced the antitumor effect (45). With regard to Shock Waves, the use of the latter has just been confirmed to permeabilize human cells and promote transfection with both cationic lipid-assembled and naked DNA (41).

Advantages, Limitations and Concluding Remarks

The studies described above suggest that Shock Waves are a promising anticancer strategy for *in vivo* applications since tissues located at different depths in the body can be readily targeted by extracorporeal treatment, with minimal histopathological damage. The ESW generator can be easily placed in contact with a water-based gel on the skin and ESW can be focused at the tumor site, thus potentially permitting to target tumor lesions. Finally, unlike ultrasound, ESW have not heating effects. This could be an advantage for *in vivo* application since temperature elevation is difficult to control spatially and temporally, especially in large tumors with heterogeneous vascularization. Unfortunately, the biggest limitation in the use of ESW in cancer therapy is that, in front of the huge amount of pre-clinical data, solid clinical trials are missing. In fact, to date, only one “old” case report combined ESW and chemotherapy to treat metastasis of prostate cancer in the iliac muscle (46), and, very recently, long-term effectiveness of ESW has been demonstrated for the treatment of lymphedema in patients with breast cancer (47).

Nevertheless, based on their multifaceted properties, the use of ESW in oncology looks promising. In fact: 1) ESW act as an "ultrasound-susceptibility modification agent“ since they may induce cell permeabilization, thus allowing better delivery of chemotherapeutic drugs into cytosol; 2) Shock Waves enhance both the cytotoxic activities of photosensitizers as well as the apoptotic signal

transduction pathway: they can act as a further tool in “sonodynamic/photodynamic” therapy; 3) Gene transfer can be induced by ESW treatment *in vivo*, particularly with enhanced acoustic cavitation, which supports the concept that “Gene and ESW therapy might be advantageously merged”; 4) Other treatment schedules are worth to be explored to evaluate the potential utility of ESW in cancer therapy, especially in combination with other modalities.

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Table 1. Different anticancer strategies using combination therapy with ESW.

Anticancer Strategies	Drugs	Models	References
Cytocidal effect of ESW	-	*In vitro* *In vivo*	4, 6, 7, 13 10, 11
ESW and chemotherapic drugs	mitomycin C; cisplatin; methotrexate; adriamycin; paclitaxel; daunorubicin; bleomycin; 5-fluouracil	*In vitro* *In vivo*	8, 9, 15, 22 16, 17
Sonodynamic Therapy	5'-aminolevulinic acid (ALA)	*In vitro* *In vivo*	23, 24 27-29
Gene Transfer	DNA plasmids; interleukin-12	*In vitro* *In vivo*	43, 44 39, 44
Nanoparticles	doxorubicin; meso-tetrakis (4-sulfonatophenyl) porphyrin (TPPS)	*In vitro* *In vivo*	32 35, 36