Review

Hyperthermia Chemo-sensitization, Chemical Thermo-sensitization and Apoptosis

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Abstract: Hyperthermia (HT) which can be directly cytotoxic to cancer cells, impairs the synthesis of cellular proteins: if not properly chaperoned by heat shock proteins (HSP), this can lead to irreversible and toxic protein aggregates. Clinically, it is preferred to use HT in combination with radiation therapy and chemotherapy. Although the combination of thermoradiotherapy has been widely studied, much attention has recently been focused on the search for compounds which can sensitize tumor cells to HT damage, but at the same time lead to minimal damage to normal cells. Synergy between HT and drugs may be caused by the occurrence of multiple events such as HT damage to ATP-binding cassette transporters, intracellular drug detoxification pathways, and the reparability of drug-induced DNA adducts. This may be why cells with acquired drug resistance (often multi-factorial) can be made responsive to drugs again by the combination of a drug exposure in conjunction with HT. In this review, the mechanism of HT-induced apoptosis and compounds which can sensitize cancer cells to HT-induced apoptosis are discussed.

Key Words: protein denaturation, protein aggregation, heat shock proteins, apoptosis

Introduction

Despite tremendous progress in chemotherapy and radiation therapy for the treatment of cancer, there is still a significant fraction of malignancies which is refractory to all current treatment modalities. The main obstacle facing conventional therapeutics is that the ‘therapeutic window’ between normal and tumor cells cannot be widened easily. An in-depth understanding of the molecular and cellular biology of cancer cells which has been made from revolutionary advances in the past two decades can now be utilized to help, provide excellent opportunities and strategies for novel therapeutic approaches. Hyperthermia has been recognized as an effective and attractive tool, especially in combination with conventional therapies, to halt tumor growth. The significant advantage of this type of combination

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therapy is the possibility of using lower doses of chemotherapy or radiation, and thus potentially widening the current ‘therapeutic windows’, leading to more effective treatment with fewer unwanted side effects, and a reduced resistance of cancer cells to the combination of drugs and radiation.

There are many studies which deal with the biochemical and biophysical mechanisms leading to cell death from HT or from a combination of HT and ionizing radiation\textsuperscript{1–5}. In this report, mechanisms are reviewed which could be involved in the enhancement of apoptosis induced by a combination of HT and drugs.

\textit{Effects of HT on cellular physiology}

Hyperthermia induces numerous changes in cellular physiology (Table I). These cellular alterations make a combination of heat and drugs very attractive. Below some important cellular responses to potential therapeutic regimens are discussed in detail.

\textbf{Table I.} Molecular effectors of hyperthermia

<table>
<thead>
<tr>
<th>Organelle</th>
<th>Functional changes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell membrane</td>
<td>Changes in fluidity/ stability</td>
<td>(65)</td>
</tr>
<tr>
<td></td>
<td>Alteration in structure</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Impairment of ion transport (Ca$^{2+}$, Na$^+$, Mg$^+$, K$^+$)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Changes in membrane potential</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Modulation of the transmembrane efflux pump</td>
<td></td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>Impairment of protein synthesis</td>
<td>(65)</td>
</tr>
<tr>
<td></td>
<td>Denaturation of protein structure and function</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aggregation of proteins</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Induction of HSP synthesis</td>
<td></td>
</tr>
<tr>
<td>Mitochondria</td>
<td>Increase permeability of mitochondrial inner membrane.</td>
<td>(67)</td>
</tr>
<tr>
<td></td>
<td>Depolarization of mitochondrial membrane potential</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Depletion of ATP production</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Production of reactive oxygen species (ROS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Disruption of Ca$^{2+}$ transport across mitochondrial membrane.</td>
<td></td>
</tr>
<tr>
<td>Endoplasmic Reticulum (ER)</td>
<td>ER stress due to excessive accumulation of misfolded proteins.</td>
<td>(68)</td>
</tr>
<tr>
<td>Nucleus</td>
<td>Impairment of RNA/DNA synthesis</td>
<td>(65)</td>
</tr>
<tr>
<td></td>
<td>Inhibition of DNA repair enzymes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alteration of DNA conformation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Changes of gene expression and signal transduction</td>
<td></td>
</tr>
</tbody>
</table>

\textit{Changes in membrane permeability}

Membranes are known to be extremely sensitive to heat stress because of their complex molecular composition of lipids and proteins. At a certain temperature, lipids change from a tightly packed gel phase to a less tightly packed crystalline phase, and permeability of the cell membrane (membrane fluidity) increases. Hyperthermia-induced cell membrane permeability leads to increased drug delivery into tumor cells. In addition, increased vascular permeability due to thermal increases in endothelial gap size also aids drug delivery into tumors. Alteration in membrane permeability also alters the cellular content of several ions (Na$^+$, Mg$^{2+}$, K$^+$) in a number of cells\textsuperscript{5}, although the changes in these ion
balances are not primarily responsible for hyperthermic cell death\(^7\)\(^{-9}\). Another ion which might be involved in hyperthermic cell death is Ca\(^{2+}\). Influxes of extracellular Ca\(^{2+}\) stimulates the activity of calmodulin-dependent protein kinases, inositol triphosphate production and other signaling cascades\(^10\).

**Alteration of cytoskeletal systems**

The response of cytoskeletal systems to HT varies depending on the cell type and HT dose\(^11\). Hyperthermia-induced disassembly of the cytoskeleton enlarges the tumor pores which enables easier drug delivery\(^11\). It also induces alteration of the mitotic spindles\(^11\), centrosome organization, and protein denaturation which results in the formation of multinucleated non-clonogenic cells\(^12\)\(^,\)\(^13\).

**Inhibition of DNA repair**

DNA appears to be the primary target for most of the currently adopted chemotherapeutic drugs\(^14\). The main obstacle in chemotherapy is the resistance of cancer cells to the cytotoxic effects of these drugs. Several possible mechanisms have been suggested to account for this. Examples of these mechanisms include intrinsic DNA repair capabilities, reduced drug diffusion into areas of vascular insufficiency, and cellular impermeability\(^15\)\(^,\)\(^16\). Hyperthermia is reported to induce DNA double strand breaks due to the denaturation and dysfunction of heat-labile repair proteins such as DNA polymerases\(^17\) or to the precipitation of denatured proteins onto nuclear chromatin structures, generating a barrier which prevents repair enzymes from reaching damage sites\(^18\). HT-induced protein denaturation is also reported to alter multiple nuclear matrix-dependent functions [e.g., DNA replication, DNA transcription, mRNA processing and DNA repair]\(^19\).

It is thus hypothesized that hyperthermic treatment sensitizes tumor cells to other cancer treatments by altering cytoskeleton reorganization, enhancing membrane permeability and inhibiting DNA repair\(^20\).

**HT-induced signal transduction**

**a) Apoptotic signal transduction**

Apoptosis is a genetically programmed and biochemically active mode of cell death in which the cell actively participates in its own destruction\(^21\). It is required for cell life span regulation and normal development\(^20\). Apoptosis also aids in the self-deletion of injured cells, terminal differentiation of epithelial cells, and organ and tissue shaping\(^21\). Abnormalities in this process are implicated in several human diseases, including cancer. Radiation, cytotoxic drugs, viruses and hyperthermia can trigger this process.

Hyperthermia within a temperature range of 41-45\(^\circ\)C induces apoptosis to varying degrees in many cell lines\(^21\). It induces apoptosis mainly through reactive oxygen species (ROS) generation, and a likely source of elevated ROS production is the mitochondria\(^22\). Mitochondria are believed to produce basal levels of ROS in the form of single-electron leakage to oxygen during normal metabolism\(^23\). This can increase dramatically under conditions in which the mitochondria are damaged or exposed to certain toxic conditions\(^22\). In addition to this, HT can also alter the expression of the Bax and Bcl-2 genes, where such changes are dependent on the sensitivity of cell lines to HT\(^24\). In thermo-resistant cell lines, HT by itself cannot change the expression of Bax and Bcl-2, but the combination of HT and
chemotherapy or radiotherapy can up-regulate Bax and down-regulate Bcl-2 expression\textsuperscript{30}. Furthermore, hyperthermia-induced increases in intracellular Ca\textsuperscript{2+} ion ([Ca\textsuperscript{2+}]) concentration is also thought to be involved in cell death. However, evidence for the role of [Ca\textsuperscript{2+}] in hyperthermic cell death is contradictory. Some investigators have stated that HT-induced increases in Ca\textsuperscript{2+} do not play a key role\textsuperscript{26}, while others have concluded that thermal perturbations in [Ca\textsuperscript{2+}] are among the primary events leading to heat-induced cell killing\textsuperscript{27–29}. These observations suggest that the role of Ca\textsuperscript{2+} in HT induced cell killing may be dependent on cell type. HT is reported to increase the expression of IP3R\textsuperscript{130} which may regulate the release of Ca\textsuperscript{2+}. Generally, Ca\textsuperscript{2+} can act on multiple targets to trigger apoptosis\textsuperscript{31}. Calpain, a calcium-dependent protease, has been considered as a possible target for calcium triggered apoptosis\textsuperscript{32}. Lipid peroxidation due to ROS generation is also reported to alter Ca\textsuperscript{2+} distribution\textsuperscript{30} and to activate a Ca\textsuperscript{2+}-dependent apoptotic pathway\textsuperscript{30} (Fig. 1).

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{hyperthermia_diagram.png}
\caption{Schematic summary of pathways involved in hyperthermic chemosensitization and chemical thermosensitization.}
\end{figure}

\textit{b) Anti-apoptosis signal transduction :}

HT also simultaneously activates signal transduction pathways leading to anti-apoptosis activity and/or cellular proliferation. Key signaling factors, such as Akt, p38, extracellular signal-regulated kinase (ERK) and heat shock proteins (HSP) play important roles in anti-apoptosis or cellular proliferation pathways\textsuperscript{34}. Such anti-apoptosis and cytoprotective signaling factors are known complications resulting from hyperthermia, and can lead to thermotolerance and chemoresistance. The presence of chemotherapeutic agents interferes with these pro-survival responses to hyperthermic monotherapy and can make cancer cells sensitive to HT.
Enhancement of hyperthermia-induced apoptosis by traditional and newly synthesized chemical agents

Hyperthermia alone plays no role in the curative treatment of human tumors\(^{35}\), therefore, much attention has been focused on combining HT with chemotherapy, and in searching for substances able to sensitize tumor cells to HT-induced damage\(^{36,37}\). An ideal sensitizer would be nontoxic at normal temperatures, but would become toxic at hyperthermic temperatures. Below, chemical agents which can act as heat sensitizers at non-toxic concentrations are briefly discussed.

a) Calcium channel blockers

Calcium channel blockers, such as verapamil or diltiazem, have been commonly used to treat arrhythmia and angina pectoris. Verapamil also has other pharmacologic actions, such as reversing multiple drug resistance and suppressing metastasis of cancer cells in BALB/c mice injected with mouse mammary adenocarcinoma F3II cells\(^{38}\). It also acts as a heat sensitizer in Chinese hamster ovary cells and U937 cells\(^{39}\). It sensitizes both normal and thermo-tolerant U937 cells to HT-induced apoptosis by increasing \([\text{Ca}^{2+}]_i\) concentration\(^{40}\).

b) Local anesthetics

Local anesthetics (LAs) belong to a class of clinically useful compounds which exert their pharmacological effect by blocking nerve impulse propagation. There are many reports showing that HT induced cell death is modified by LAs (e.g. potentiation by procaine in murine LS178Y lymphoma cells\(^{41}\), by lidocaine in murine FM3A mammary carcinoma cells\(^{42}\) and in murine tumor models\(^{43}\), and by dibucaine, tetracaine, and procaine in hepatoma tissue culture cells\(^{44}\)). Investigations of the molecular mechanism of apoptosis enhancement by LAs at hyperthermic temperatures, have produced evidence that the elevation of \([\text{Ca}^{2+}]_i\) is due to increased releases of \(\text{Ca}^{2+}\) from intracellular storage sites, and that this is caused by HT along with increased activity of the mitochondria caspase-dependent pathway (partly regulated by \([\text{Ca}^{2+}]_i\)), and that this plays a crucial role in the enhancement of apoptosis induced by the combination of HT and lidocaine\(^{45}\).

c) Temperature-dependent free radical generator

A temperature-dependent free radical generator, 2,2’-azobis (2-amidinopropane) dihydrochloride (AAPH) acts as a heat sensitizer at non-toxic concentrations. It can sensitize hypoxic and thermo-tolerant cells, and enhances HT-induced apoptosis by increasing the \([\text{Ca}^{2+}]_i\) concentration. Hyperthermia combined with exposure to AAPH led to a loss of mitochondrial membrane potential (MMP), indicating that mitochondria function was damaged by this treatment\(^{30}\). Furthermore, AAPH has been reported to inhibit HT-induced anti-apoptosis signaling factors\(^{46}\).

d) Intracellular \(\text{H}_2\text{O}_2\) generator

Another chemical agent which acts as a thermosensitizer is 6-formylpterin (6-FP). This compound generates intracellular \(\text{H}_2\text{O}_2\) by transferring electrons from NAD(P)H to oxygen\(^{47}\), and at nontoxic concentrations, it enhances HT-induced apoptosis. Some investigations suggest that the increase in the \([\text{Ca}^{2+}]_i\) concentration, the activation of mitochondria-caspase dependent pathways, and the translocation of PKC\(\delta\) to mitochondria play important roles in 6-FP\(^{48}\) enhancement of HT-induced apoptosis.

e) Spin trap: \(\alpha\)-phenyl-tert-butyl nitrate

Nitrate spin trap, \(\alpha\)-phenyl-tert-butyl nitrate (PBN) has been used, not only for electron
paramagnetic resonance (EPR) -spin trapping studies, but also as an antioxidant in vitro and in vivo because PBN reacts with oxygen radicals to produce less reactive species\textsuperscript{49,50}. It has been reported that nitric oxide (NO) is released from PBN under oxidative stress conditions\textsuperscript{51,52}, and that HT induces oxidative stress in cells due to generation of superoxide (O$_2^-$). Consistent with these observations, it has been observed that PBN acts as a sensitizer for HT-induced apoptosis. When U937 cells were treated with a combination of HT and PBN, a significant enhancement of apoptosis was observed because NO reacts with O$_2^-$ and produces ONOO$^-$, and this ROS enhances HT-induced apoptosis via stimulation of the mitochondria-caspase and the [Ca$^{2+}$]i -dependent pathways\textsuperscript{53}.

\textbf{f) Furan-fused tetracyclic compounds}

Recently, it was reported that a newly synthesized furan-fused tetracyclic compound, DF3 (a triisopropylsiloxy (TIPS) derivative) acts as a heat sensitizer in U937 cells. DF3, at nontoxic concentrations with HT (44°C for 20 min), showed a significant enhancement of heat-induced apoptosis. DF3 induced a sustained elevation of O$_2^-$ and a transient rise of H$_2$O$_2$ generation, and there was a further increase in these compounds when DF3 exposure was combined with HT. This enhancement in oxidative stress appears to be the key event in the improved apoptotic response of U937 cells when subjected to a combination of DF3 and HT\textsuperscript{54}.

\textit{Mild hyperthermia enhances chemical agent induced apoptosis}

Hyperthermia produces synergistic results when combined with radiation or cytotoxic drugs at lower temperatures (40.5-43°C) (Table II). However, it is beyond the scope of this short review to discuss all of the observed interactions between these drugs and HT separately. Below is a summary of reports describing some chemical agents which showed a synergistic enhancement of apoptosis when combined with mild HT.

\textit{a) DNA-damaging agents}

HT can enhance the cytotoxicity of various DNA damaging agents. Most impressive is data for a combined HT and cisplatin treatment. Hyperthermia enhances intra-cellular platinum accumulation, reduces intra-cellular detoxification which leads to more platinum-induced DNA adducts, and inhibits repair of these adducts\textsuperscript{55}. Interestingly, all of these processes are up-regulated in cells which have acquired platinum resistance\textsuperscript{56}. All of these enhancement effects appear to be due to HT-induced changes in cell metabolism, excretion, and membrane permeability or membrane transport\textsuperscript{56}. This has led to the suggestion that hyperthermia could be instrumental in reversing drug resistance, a major clinical problem in chemotherapy.

\textit{b) Macrophelides}

Recently, it was reported that one of the synthetic diketone macrophelides, specifically MS5 which is a 16-membered macrolide compound\textsuperscript{57}, led to the synergistic enhancement of apoptosis when combined with mild HT (41°C for 20 min) in U937 lymphoma cells. Significant increases in reactive oxygen species (ROS) generation were observed immediately after the combined treatment. Combination of MS5 and mild HT also led to a MMP loss, indicating that mitochondrial dysfunction occurred during this treatment. Furthermore, Fas expression, caspase-8 and caspase-3 activation were also observed at significantly increased levels after the combined treatment when compared with mild HT treatment alone.
Table II. Heat-induced enhancement of drug cytotoxicity in apoptosis

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Target for apoptosis</th>
<th>Cell line</th>
<th>Concentration/°C</th>
<th>Maximum Enhancement Ratio</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin (DNA damaging agent)</td>
<td>DNA</td>
<td>SW1573</td>
<td>5 μM/41-43°C (60 min)</td>
<td>7.3-7.8* (69)</td>
<td></td>
</tr>
<tr>
<td>Lobaplatin (DNA damaging agent)</td>
<td>DNA</td>
<td>SW1573</td>
<td>10 μM/41-43°C (60 min)</td>
<td>1.6-2.9* (69)</td>
<td></td>
</tr>
<tr>
<td>Oxaliplatin (DNA damaging agent)</td>
<td>DNA</td>
<td>SW1573</td>
<td>10 μM/41-43°C (60 min)</td>
<td>2.0-2.1* (69)</td>
<td></td>
</tr>
<tr>
<td>Paclitaxel (Microtubule stabilizing agent)</td>
<td>Microtubules</td>
<td>FM3A</td>
<td>10 μM/43°C (60 min)</td>
<td>2.1* (70)</td>
<td></td>
</tr>
<tr>
<td>Etoposide (VP-16) (DNA damaging agent)</td>
<td>DNA</td>
<td>LU65A</td>
<td>8 μM/43°C (45 min)</td>
<td>1.8* (71)</td>
<td></td>
</tr>
<tr>
<td>5-Fluorouracil (Anti-metabolite)</td>
<td>DNA and RNA</td>
<td>CCRF-CEM</td>
<td>100 μM/42°C (120 min)</td>
<td>1.0* (72)</td>
<td></td>
</tr>
<tr>
<td>Verapamil (Ca** Channel blocker)</td>
<td>Mitochondria</td>
<td>U937</td>
<td>100 μM/42-44°C (30 min)</td>
<td>1.9-4.1 i (37)</td>
<td></td>
</tr>
<tr>
<td>Lidocaine (Local anesthetic)</td>
<td>Mitochondria</td>
<td>U937</td>
<td>1 mM/44°C (10 min)</td>
<td>3.6 i (44)</td>
<td></td>
</tr>
<tr>
<td>2-Amidinopropyl dihydrochloride (Temperature dependent free radical generator)</td>
<td>Unknown</td>
<td>U937</td>
<td>50 μM/44°C (10 min)</td>
<td>5.0 i (29)</td>
<td></td>
</tr>
<tr>
<td>6-Formylpterin (Intracellular hydrogen peroxide generator)</td>
<td>Mitochondria</td>
<td>U937</td>
<td>300 μM/44°C (20 min)</td>
<td>4.4 i (49)</td>
<td></td>
</tr>
<tr>
<td>α-phenyl-tert-butyl nitrosyl (Spin trap agent, antioxidant)</td>
<td>Unknown</td>
<td>U937</td>
<td>10 mM/44°C (10 min)</td>
<td>5.7 i (54)</td>
<td></td>
</tr>
<tr>
<td>Furan-fused tetracyclic compounds (Anti-viral agent)</td>
<td>Mitochondria</td>
<td>U937</td>
<td>20 μM/44°C (20 min)</td>
<td>6.6 i (55)</td>
<td></td>
</tr>
<tr>
<td>Macrophelides (Anti-metastatic agent)</td>
<td>Mitochondria</td>
<td>U937</td>
<td>5 μM/41°C (20 min)</td>
<td>2.1 i (57)</td>
<td></td>
</tr>
<tr>
<td>Anisomycin (Protein synthesis inhibitor)</td>
<td>Proteins</td>
<td>U937</td>
<td>0.1 μM/41°C (60 min)</td>
<td>1.5 i</td>
<td></td>
</tr>
<tr>
<td>6-Dimethyl-a-minopurine (Protein kinase inhibitor)</td>
<td>Unknown</td>
<td>U937</td>
<td>2 mM/41°C (60 min)</td>
<td>1.5 i</td>
<td></td>
</tr>
</tbody>
</table>

*Maximum enhancement ratio = Cell death (%) (measured with different methods) in the presence of the drug at elevated temperature/Cell death (%) in the presence of drug at a normal temperature (37°C).
‡ Maximum enhancement ratio = DNA fragmentation (%) in the presence of a drug at elevated temperatures/DNA fragmentation (%) in the presence of a drug at a normal temperature (37°C).
# Unpublished data.
Moreover, this combined treatment also altered the expression of apoptosis-related proteins, which was shown by the cleavage of Bid and the down-regulation of Bcl-2. The results have indicated that an early increase in ROS generation was primarily responsible for the synergistic enhancement of apoptosis after a combined treatment\textsuperscript{58}.

c) **Anisomycin**

The effects of HT in combination with antibiotics were reported previously\textsuperscript{43}. Some antibiotics such as Bleomycin, Doxorubicin and Actinomycin D showed more than an additive cell killing when combined with HT\textsuperscript{35}.

Recently, the effect of a combination of mild HT (41°C for 60 min) and anisomycin was examined in U937 cells. This antibiotic is a reversible inhibitor of protein biosynthesis and also has been reported to inhibit DNA synthesis\textsuperscript{59}. It also induces strong activation of JNK and P38 mitogen-activated protein kinase\textsuperscript{60}. In preliminary studies, a significant synergistic enhancement of apoptosis was observed after a combined treatment when compared to anisomycin alone (27.2±3.0% and 17.8±4.1% respectively). Mitochondrial dysfunction was also observed during this treatment indicating the involvement of mitochondrial apoptosis pathways.

d) **6-Dimethylaminopurine**

Many alkylating agents, such as Melphalan, Ifosfamide and Mitomycin C\textsuperscript{57} have been reported to show additive cell killing when combined with HT. The alkylpurine, 6-dimethylaminopurine (6-DMAP) is a protein kinase inhibitor known to induce premature mitosis in S-phase arrested hamster cells\textsuperscript{61,62}, and has been reported to induce apoptosis in HeLa cells\textsuperscript{63} and in epidermal growth factor and insulin treated G\textsubscript{2}-phase Chinese hamster embryonic fibroblast (CHEF/18) cells\textsuperscript{64}. The effects of 6-DMAP in combination with mild HT (41°C for 60 min) were examined in U937 cells. Preliminary data showed a synergistic enhancement of DNA fragmentation in cells treated with the combination when compared to 6-DMAP and mild HT alone (43.4±8.2%, 28.6±6.2% and 8.4±0.4% respectively). However, further studies with anisomycin and 6-DMAP in combination with HT are still necessary to propose any detailed mechanism which can explain how these agents may be involved with apoptosis.

**Conclusion**

HT sensitizes cells to many cytotoxic agents, and even converts some innocuous drugs into highly toxic agents. HT alters the pharmacokinetics and pharmacodynamics of drugs\textsuperscript{55}, increases DNA damage\textsuperscript{55}, decreases DNA repair\textsuperscript{6,12} and alters membrane permeability\textsuperscript{65}. Furthermore, HT can also lead to the modulation of cellular ROS concentrations, which could be responsible for thermal chemo-sensitization in cancer therapy. These HT-induced cellular changes may also have the potential to overcome some types of drug resistance\textsuperscript{66}. Further detailed studies with combinations of drugs and HT are still required to identify effective heat sensitizers for cancer therapy.

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References
41) Yau T.M.: Procaine-mediated modification of membranes and of the response to X-irradiation and hyperthermia in


