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Treating cancer with heat: Hyperthermia as promising strategy to enhance apoptosis

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REVIEW ARTICLE

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Abstract

The fundamental idea and the effects of heat on cancer cells are well known. However, the results obtained in therapy by hyperthermia (HT) alone have been only partially satisfactory. Treatment at temperatures between 40 and 44°C is cytotoxic for cells in an environment with a low oxygen partial pressure and low pH, conditions that are found specifically within tumour tissue, due to insufficient blood perfusion. Under such conditions radiotherapy is less effective, and systemically applied cytotoxic agents will reach such areas in lower concentrations than in well-perfused areas. Therefore, clinically, it is preferred to use hyperthermia in combination with radiation therapy and chemotherapy. Hyperthermia can be applied by several methods: local hyperthermia by external or internal energy sources; regional hyperthermia by perfusion of organs or limbs, or by irrigation of body cavities; and whole-body hyperthermia. Number of studies have reported the combination of thermo-radiotherapy. Consequently, much attention has been focussed on identifying agents among the conventional chemotherapeutic substances that can sensitise tumour cells to hyperthermia-induced damage with minimal effects on normal cells. In this review, we overviewed important mechanisms of hyperthermia-induced apoptosis and the substances which can act as heat sensitisers in cancer therapy.

Keywords: Hyperthermia, Apoptosis, Heat sensitizer.

Introduction

"Those who cannot be cured by medicine can be cured by surgery. Those who cannot be cured by surgery can be cured by fire [hyperthermia]. Those who cannot be cured by fire, they are indeed incurable".—Hippocrates (479–377)

The use of elevated temperature, hyperthermia (HT) is not a new treatment for cancer. Hippocrates was aware of the potential of heat to cure or shrink tumours. Tumour shrinkage after a high fever due to an infection was reported in 1866.[1] Heat has profound effects on cells. At low doses, heat enhances recovery from injury. At high doses, it leads to cell death that may be immediate for extreme doses. The role of HT alone as a cancer treatment is limited. It has been recognised as an effective and useful tool, especially in combination with conventional therapies to halt tumour growth. The significant advantage to this type of combination therapy is the possibility of using lower doses of chemotherapy and radiation leading to more effective treatment with fewer unwanted side effects, and a reduced resistance of cancer cells to the combination of drug and radiation. Numerous clinical trials have studied hyperthermia in combination with conventional therapies. Many of these studies, but not all, have shown a significant reduction in tumour size when hyperthermia is combined with other treatments.[2] Temperature between 41°C-44°C is not toxic to the normal cells, but show toxicity in cancerous cells.

In the clinical application of HT, three methods can be employed: local, regional and whole-body HT. The choice of the method to use on any given patient depends on the type of the cancer, its location and its stage. Special thermometres are used to monitor the temperature during the course of the treatment.

Local HT is used to treat localised cancers. It can be applied by external, intraluminal and interstitial methods to treat tumours on the skin or just underneath it, tumours within or near the body cavities, and to treat deep tumours within the body, such as brain tumour. Different types of energy sources may be used to apply heat such as microwave, radiofrequency and ultrasound.[3]

Regional HT is used to heat large areas of tissue such as body cavity, organ or limb. Different methods can be employed depending on the type of cancer. For example, deep tissue regional HT is applied for cancers within the body like bladder[4] or cervix; regional perfusion technique is applied for the cancers of arm and legs such as melanoma or cancers of some organs like liver or lung; peritoneal perfusion technique is applied for the treatment of cancers within the peritoneal cavity, including primary peritoneal mesothelioma and stomach cancer.[3]

Whole-body HT is used to treat metastatic cancers. A common characteristic is that energy is introduced into

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the body while at the same time energy losses are minimised. Most recent heat induction methods used are irradiation with infrared, radiofrequency and microwave electromagnetic energy. These techniques can be applied solely or in combination.[3]

Normal tissue toxicity will result directly from hyperthermia when the tolerance limits are exceeded. Experimental studies have shown that most normal tissues are not damaged when the temperature over 1 hour of treatment does not exceed 44°C. The toxicity from superficial hyperthermia is usually a skin burn, heals with conservative treatment. Subcutaneous fat burns were seen in 3%-12% of the patients treated with deep hyperthermia. It does not usually cause much discomfort to the patient who feels a subcutaneous lump, which is tender for a few days to a maximum of a few weeks and then disappears spontaneously. Toxicity from whole-body hyperthermia depends on, besides temperature, the patient's general condition, condition of organ systems and the physiological conditions during the treatment. During any application of hyperthermia, it is important to avoid pressure sites, since hypoxic normal tissues will be more sensitive to hyperthermia.[3]

HT is known as a potent radio-sensitiser and 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 ionising radiation. Studies are also available on the combination of HT and drugs-induced apoptosis. In this review, we will discuss the factors and mechanisms which make combination of HT with drugs and radiation an effective tool for cancer therapy.

Cellular physiological changes induced by HT

Hyperthermia induces numerous changes in cellular physiology (Table-1). These cellular alterations make a combination of heat with drugs and radiation very effective. Below some important cellular responses to potential therapeutic regimens are discussed in detail:

Alteration 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 tumour cells. In addition, increased vascular permeability due to heat increases in endothelial gap size also aids drug delivery into the tumours. Alteration in membrane permeability also alters the cellular content of several ions (Na^+, Mg^{2+}, K^+) in a number of cells, although the changes in these ion balances are not primarily responsible for hyperthermic cell death.[5] Another ion which might be involved in hyperthermic cell death is Ca^{2+}. Influxes of extracellular Ca^{2+} stimulate the activity of calmodulin-dependent protein kinases, inositol triphosphate production and other signalling cascades.[6]

Table-1: Molecular effectors of hyperthermia.[35]

Cellular components	Functional alterations
Cell membrane	Alterations in fluidity/ stability
	Changes in structure
	Impairment of ion transport (Ca^{2+}, Na^+, Mg^+, K^+)
	Modification in membrane potential
	Modulation of the transmembrane efflux pump
Cytoplasm	Denaturation of protein structure and function
	Impairment of protein synthesis
	Aggregation of proteins
	Induction of HSP synthesis
Mitochondria	Amplification of mitochondrial inner membrane permeability.
	Depolarization of mitochondrial membrane potential
	Reduction of ATP production
	Generation of reactive oxygen species (ROS)
	Disruption of Ca^{2+} transport across mitochondrial membrane.
Endoplasmic Reticulum (ER)	ER stress due to excessive accumulation of misfolded proteins.
Nucleus	Impairment of RNA/DNA synthesis
	Inhibition of DNA repair enzymes
	Alteration of DNA conformation
	Modification of gene expression and signal transduction.

RNA: Ribonucleic acid.
DNA: Dioxyribonucleic acid.

Modification of cytoskeletal system

The response of cytoskeletal systems to HT varies depending on the cell type and HT dose.[7] Hyperthermia-induced disassembly of the cytoskeleton, enlarges the tumour pores which enables easier drug delivery.[7] It also induces alteration of the mitotic spindles,[7] centrosome organisation, and protein denaturation which results in the formation of multinucleated non-clonogenic cells.[8] Furthermore, it also induces alterations of integrin-cytoskeleton network with concomitant cell shape change, anoikis and programmed cell death.[9]

Inhibition of DNA repair

Dioxyribonucleic acid (DNA) appears to be the primary target for most of the currently adopted chemotherapeutic drugs and radiation.[10] The main obstacle for cancer treatment is the resistance of cells to the cytotoxic effects of drugs and radiation. Several possible mechanisms have been suggested to account for this resistance, for example, intrinsic DNA repair capabilities, vascular insufficiency, and cellular impermeability.[11]

Hyperthermia is reported to induce DNA double-strand breaks due to the denaturation and dysfunction of heat-labile repair proteins such as DNA polymerases[12] or to the precipitation of denatured proteins onto nuclear chromatin structures, generating a barrier which prevents repair enzymes from reaching damage sites.[13] HT-induced protein denaturation is also reported to alter multiple nuclear matrix-dependent functions [e.g., DNA replication, DNA transcription, Messengar ribonucleic acid (mRNA) processing and DNA repair].[14]

It is thus hypothesised that hyperthermia treatment sensitises tumour cells to chemotherapeutic drugs or radiation by altering cytoskeleton re-organisation, enhancing membrane permeability and inhibiting DNA repair.[15]

HT-induced signalling pathways

Pro-apoptotic signaling pathways

Apoptosis is a genetically programmed and biochemically active mode of cell death in which the cell actively participates in its own destruction.[16] It is required for cell life span regulation and normal development.[15] Apoptosis also aids in the self-deletion of injured cells, terminal differentiation of epithelial cells, and organ and tissue shaping.[16] 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°C induces apoptosis to varying degrees in many cell lines.[17] It induces apoptosis mainly through reactive oxygen species (ROS) generation, and a likely source of elevated ROS production is the mitochondria.[17] Mitochondria are believed to produce basal levels of ROS in the form of single-electron leakage to oxygen during normal metabolism.[18] This can increase dramatically under conditions in which the mitochondria are damaged or exposed to certain toxic conditions.[17] In addition, 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.[19] 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.[20] Furthermore, hyperthermia-induced increase in intracellular Ca^{2+} ion ($[Ca^{2+}]i$) concentration is also thought to be involved in cell death. However, evidence for the role of $[Ca^{2+}]i$ in hyperthermic cell death is contradictory. Some investigators have stated that HT-induced increase in Ca^{2+} does not play a key role,[21] while others have concluded that thermal perturbations in $[Ca^{2+}]i$ are among the primary events leading to heat-induced cell killing.[22] These observations suggest that the role of Ca^{2+} in HT-induced cell killing may be dependent on cell type. HT is reported to increase the expression of the receptor inositol triphosphate[23] which may regulate the release of Ca^{2+}. Generally, Ca^{2+} can act on multiple targets to trigger apoptosis.[24] Lipid peroxidation due to ROS generation is also reported to alter Ca^{2+} distribution[25] and to activate a Ca^{2+}-dependent apoptotic pathway[23] (Figure).

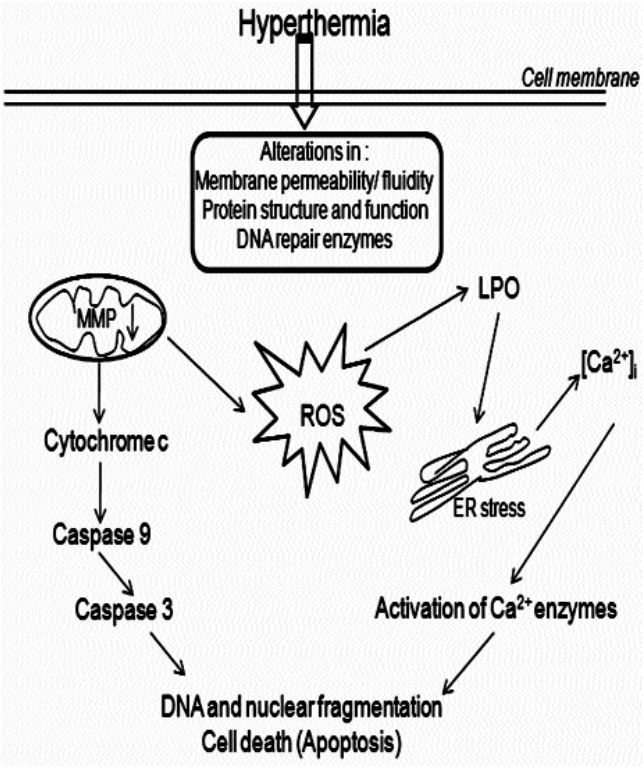

Figure: Scheme of pro-apoptotic signalling pathways activated by hyperthermia.

Anti-apoptotic signaling pathways

HT is also responsible to activate survival/antiapoptotic pathways that lead to the development of thermo tolerance. Signalling factors, such as Akt, p38, extracellular signal-regulated kinase (ERK) etc play important roles in anti-apoptosis or cellular proliferation pathways.[26] Such anti-apoptosis and cytoprotective signalling factors are negative for hyperthermic cancer therapy. Therefore, targeted inhibition of the anti-apoptotic signalling pathways is an attractive approach for the development of hyperthermia-induced chemo-sensitisation.

Heat shock proteins (HSPs)

Heat shock proteins (HSPs) were originally identified as proteins whose expression was markedly increased by heat shock.[26] HSPs represent a heterogeneous group of molecular chaperones consisting of at least five sub-groups with different molecular mass and partially varying biological function. HSPs are usually divided into small HSPs

(molecular mass <40 kDa), and the HSPs60, HSPs70, HSPs90 and HSPs100 proteins families.[26] All HSPs families share their chaperoning function, i.e. they unselectively bind to hydrophobic protein sequences liberated by denaturation. Thus they prevent irreversible interaction with neighbour proteins that would result in a loss of function. Several HSPs are expressed even in unstressed cells and play important function in normal cell physiology.[26] HSPs synthesis can be induced within minutes by activation of so-called 'heat shock factors'(HSF). In hyperthermia, HSPs are thought to be involved in the protection of cells against heat damage.

Hyperthermia and drug resistance

Drug resistance represents the major cause of treatment failure in human malignancies, and can be induced by different mechanisms, of which the pleitrope multidrug resistance (MDR), has gained particular interest.[27] Hyperthermia is a good candidate to overcome various modes of drug resistance. A number of phase I and II trials reported successful treatments of patients with chemorefractory tumours by adding HT to antineoplastic chemotherapy.[28] On the other hand, moderate heat exposure has been demonstrated to induce HSP expression in cultured cells, and elevated levels of intracellular HSP 70 have been shown to be associated with thermotolerance which may be linked with different forms of drug resistance (e.g. MDR), and HT has been shown to induce various forms of drug resistance, too (including MDR or the heat-dependant inactivation of the enzyme topoisomerase II). Reversal of drug resistance has been particularly shown for platinum compounds at temperatures >42°C, whereas induction of drug resistance (alone or in conjunction with thermotolerance and HSP-accumulation) may appear when lower temperatures are applied. However, available data on hyperthermia and drug resistance suggest that the positive effect (reversal of drug resistance) overweighs the disadvantages (induction of drug resistance) in clinical practice.[29]

Hyperthermia-induced radiosensitisation

Hyperthermia and radiation act in a synergistic way. This synergism induces an increase in cell killing even at lower temperatures, which is not the case when hyperthermia is implemented alone.[29] It appears most pounced in S-phase cells that are usually resistant to radiation alone. Hypoxic cells, as well as, those with impaired nutrient supply and/or acidic pH have been shown to react very sensitively to the combined treatment of heat and radiation.[30]

Hyperthermia-induced chemosensitisation

Synthetic heat sensitisers:

One of the promising approaches to the clinical application of HT is to combine it with chemotherapy ('thermochemotherapy'). Numerous in vitro and in vivo systems have been used to demonstrate the synergistic effects of HT when combined with a wide range of chemotherapeutic agents. The combined treatments have shown increased success in many tumours.[31] The synergistic effects occur due to the alteration in the pharmacokinetics[32] and pharmacodynamics of drugs,[29] increases DNA damage[11] and decreases DNA repair.[5,8] It converts some innocuous drugs into highly toxic agents the so-called 'heat sensitisers/thermosensitisers.' An ideal heat sensitiser would be non-toxic at normal temperatures, but become toxic at hyperthermic temperature. There are chemical agents that can act as heat sensitisers at non-toxic concentrations (Table-2).

Natural heat sensitisers

Natural compounds contain greater characteristics of high chemical diversity and biochemical specificity than standard chemical compounds. Several studies have reported the role of natural compounds in cancer cell apoptosis.[33] However, quercetin is the only bioflavonoid reported in combination with hyperthermia as an

Table-2: Synthetic compounds as a heat sensitiser.[35]

Drugs	Cell line	Concentration/°C	Target for apoptosis	Maximum Enhancement Ratio
Cisplatin (DNA damaging agent)	SW1573	5 M /41-43°C (60min)	DNA	7.3 - 7.8 *
Paclitexal (Microtubule stabilizing agent)	FM3A	10 M /43°C (60min)	Microtubules	2.1 *
Etoposide (VP-16) (DNA damaging agent)	LU65A	8 M /43°C (45min)	DNA	1.8 *
5-Fluorouracil (Anti-metabolite)	CCRF-CEM	100 M / 42°C (120min)	DNA and RNA	1.0 *
Verapamil (Ca2+ Channel blocker)	U937	100 M /42-44°C (30min)	Mitochondria	1.9 - 4.1 §
Lidocaine (Local anesthetic)	U937	1 mM /44°C (10min)	Mitochondria	3.6 §
6-Formylpterin (Intracellular hydrogen peroxide generator)	U937	300 M /44°C (20min)	Mitochondria	4.4 §
Furan-fused tetracyclic compounds (Anti-viral agent)	U937	20 M /44°C (20min)	Mitochondria	6.6 §
Macrosphelides (Anti-metastatic agent)	U937	5 M /41°C (20min)	Mitochondria	2.1 §
Anisomycin (Protein synthesis inhibitor)	U937	0.1 mM/ 41°C (60min)	Proteins	1.5 §

DNA: Dioxyribonucleic acid. *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).

inhibitor of HSP 70.[34] Numbers of medicinal plants are present which can be studied with HT in order to identify effective heat sensitisers for cancer therapy.

Conclusion

The clinical application of HT has a strong biological rationale. Still research is going on to overcome the technical problems associated with this therapy. Resistance to conventional anti-cancer drugs creates a dire need to apply alternative strategies like HT in treating cancers. Furthermore, combination of HT with available chemotherapeutic agents or with novel candidates from natural source might open new dimensions in targeting resistant cancers. Further elucidation of the molecular mechanisms responsible for the enhancement of heat-induced apoptotic cell death may provide a strong basis for the effective use of HT and HT-induced apoptosis sensitisers in cancer therapy.

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