AN INNOVATIVE THERAPEUTIC APPROACH IN ONCOLOGY: HYPERThERMIA

Mehmet TOPÇUL1*, İdil ÇETİN2

1* İstanbul University, Faculty of Science, Department of Biology, Istanbul/Turkey. 
2İstanbul University, Institute of Science, Department of Radiobiology, Istanbul/Turkey.

Author’s Corresponding Address:

1*Mehmet TOPÇUL
Istanbul University, Faculty of Science, Department of Biology, 34459, Vezneciler, Istanbul/Turkey.
E-mail: topcul@istanbul.edu.tr

İdil ÇETİN
Istanbul University, Faculty of Science, Department of Biology, 34459, Vezneciler, Istanbul/Turkey.
E-mail: idil.cetin@istanbul.edu.tr

ABSTRACT
Failing in the cure of cancer sometimes by conventional treatment methods points us to make new approaches besides these conventional methods. Using hyperthermia which effects angiogenesis and cancer stem cells, combination with chemotherapy, radiotherapy and gene therapy is seen as a method that can help the treatment of cancer.

Key words: Hyperthermia, Radiotherapy, Chemotherapy, Angiogenesis, Gene Therapy, Cancer Stem Cells

Introduction
The therapeutic effects of hyperthermia on various types of malignant diseases have been recognized for more than 100 years (Luk et al., 1980). Hyperthermia is an emerging effective treatment method in oncology. It has became a new modality of cancer treatments, showing significant improvements in tumor response rates and patient morbidity in combination with other treatment methods, such as surgery, chemotherapy, radiation therapy and gene therapy or applied as a single therapy. Nevertheless, hyperthermia is still in its infancy (Fiorentini and Szasz, 2006). Hyperthermia is a therapeutic procedure used to raise the body or local tissue temperature to about 41-43°C through the application of electromagnetic or ultrasound energy for a defined period of time to sensitize cells for additional therapies (Cabuy, 2011).
Depending on the organ bearing the cancerous tissue, stage of cancer development and method of energy delivery to patient’s body, three kinds of hyperthermia techniques are recognized. This brings about various equipments and treatment works. These types are local hyperthermia, regional hyperthermia and whole-body hyperthermia (Serdari and Verga, 2011).

Numerous clinical trials have studied hyperthermia in combination with radiation therapy and/or chemotherapy. These studies have focused on the treatment of many types of cancer, including sarcoma, melanoma, and cancers of the head and neck, brain, lung, esophagus, breast, bladder, rectum, liver, appendix, cervix, and peritoneal lining (mesothelioma) (van der Zee, 2002; Wust et al., 2002; Falk and Issels, 2001; Dewhirst et al., 2000 et al., 2000). Despite difficulties in increasing human tumor temperatures, recent clinical trials have shown that a combination of hyperthermia with radiation is superior to radiation alone in controlling many human tumors (Dewey et al., 1977; Gabriele and Roca, 2006). The increased effect seen by combining cytotoxic agents with hyperthermia is complex, but may be due to altered drug pharmacokinetics such as increased solubility (e.g. nitrosureas and alkylating agents), altered plasma protein binding (e.g. cisplatinum) and activation of enzymatic processes (e.g. anthracyclines) (Luk et al., 1980; Gerweck, 1985). During the last two decades, hyperthermia has been used as an efficient complement to standard cancer treatments such as radiation therapy and chemotherapy. A further advantage is that hyperthermia can eliminate drug-resistant and radio-resistant tumour cells (Bettaieb and Wrzal, 2013).

The main actions of hyperthermia in the neoplastic tissues are the following: Greater heat sensitivity of neoplastic tissues to hyperthermia, due to its chronic ischemia, hypoxia and acid pH; lethal effect of temperature of 42-43 °C on tumor cells, depending on the application time; temporary growth stabilization of tumor cells after a moderate hyperthermia (39-41 °C); prolonged action of temperature, due to lower thermal dissipation, caused by a chronic ischemia inside the tumor, as a result of its reduced vessel regulation mechanisms; alterations in the neoplastic cell cycle, which lead to the blocking of mitosis, due to a disruption in the S phase; marked action on the core of the tumor, less sensitive to radiation because of ischemia, hypoxia and low pH; action in favor of apoptosis mechanisms (Donkol and Nammi, 2013).

Hyperthermia causes many changes in cells and leads to a loss of cellular homeostasis. A key event appears to be protein denaturation and aggregation, which results in cell cycle arrest, inactivation of protein synthesis, and inhibition of DNA repair processes. Other cellular effects of hyperthermia include: (1) the inhibition of DNA synthesis, transcription, RNA processing and translation; (2) increased degradation of aggregated/misfolded proteins through the proteasomal and lysosomal pathways; (3) disruption of the membrane cytoskeleton; (4) metabolic changes (e.g. uncoupling of oxidative phosphorylation) that lead to decreased levels of ATP; and (5) alterations in membrane permeability that cause increases in intracellular levels of Na⁺, H⁺ and Ca²⁺ (Bettaieb and Wrzal, 2013).

Elevated temperatures can increase the rates of biochemical reactions and this would increase cell metabolism, which should cause increased oxidative stress. Levels of reactive oxygen species (ROS) were shown to increase after exposure to both lethal (≥42°C) and non-lethal (40°C) temperatures (Bettaieb and Wrzal, 2013).

Various targets in the cell affected by rises in temperature have been found, such as membranes, the cytoskeleton, synthesis of macromolecules, and DNA repair (Wust et al., 2002). Hyperthermia has different effects in cellular, immunological and molecular biological levels. These effects can be considered as cytotoxic effects such as induction of apoptosis, synergistics with cytostatics and ionized rays, higher transmembranic carrier mechanism (tissue penetration, permeability), increased metabolism, reduction of chemotherapy-resistancy, reduction of intratumoural tissue pressure; immunological effects such as improvement of emigration migration and phagocytosis, induction of cytokines, chemokines and heat-shock proteins, modulation of cell-adhesions molecules, higher extravasation and permeability for antibodies;
molecular-biological effects such as antiangiogenic and antivascular effects induction of heat-shock proteins, expression of antigens and modification of gen-expression (Hager et al., 2006).

In this review, we discussed about therapeutic effects of hyperthermia combination with chemotherapy, radiotherapy and gene therapy and also the effects of hyperthermia on angiogenesis which is an important mechanism for tumor growth and cancer stem cells which are responsible for tumor recurrence.

**Hyperthermia combination with chemotherapy**

The efficiency of chemotherapeutic as well as radiotherapeutic cancer treatment is generally restricted by the expression of multidrug resistance (MDR) transporters that confer resistance to a variety of structurally unrelated, clinically important antineoplastic agents (Gottesman et al., 2002; Fojo et al., 1987; Kvackayová-Kisucká et al., 2001). Multidrug resistance (MDR) is characterized by cross-resistance to four classes of commonly used anticancer drugs such as Vinca alkaloids, anthracyclines, taxanes, and epipodophyllotoxins (Bettaieb and Wrzal, 2013).

Heat modifies the cytotoxicity of many chemotherapeutic agents (Issels, 2008). Three types of drug interactions have been categorized. The first are drugs that show an increase in effectiveness with increased temperature, even those below 42°C. The second category of drugs seem to show increased effectiveness only above a threshold temperature value. The third category are those drugs that are normally not considered of any value therapeutically at 37°C but that show significant killing ability at elevated temperatures (Perez et al., 1989).

The thermal enhancement ratios (TER) which represent the pharmacodynamic features of the drug-heat interaction were determined for different antineoplastic agents including Cisplatin, Cyclophosphamide, Ifosfamide, Ifosfamide, Melphalan, BCNU, Bleomycin, Mitomycin C, 5-Fluorouracil, Doxorubicin (Issels, 2008). The mechanisms involved in the interaction of chemotherapy and hyperthermia are depended on the particular chemotherapeutic agent and thought to be related to an increase in drug uptake, expand of DNA damage and inhibition of DNA repair (Istomin et al., 2008).

**Hyperthermia combination with radiotherapy**

Tumor cell environment, such as hypoxia, poor nutrition, and low pH, while detrimental to cell kill by ionizing radiation, is beneficial to heat therapy. Acidic environment of tumor confers resistance to radiation but favors cell kill due to heat. The effect of hyperthermia depends on the temperature and exposure time (Serdari and Verga, 2011). Several mechanisms are responsible for the supra-additive effect of the combination of radiotherapy and hyperthermia. The additive effect comes from the sensitivity of cells in the hypoxic, low pH areas, and the cells in the S-phase, which are both relatively radioresistant (Raaphorst, 1990). Hyperthermia may cause an increased blood flow, which may result in an improvement in tissue oxygenation, which then results in a temporally increased radiosensitivity (Zee, 2002).

Experimental studies have shown that hyperthermia- artificial raising of temperature to 40-45°C is an effective method of killing cells, especially for cells in hypoxic, nutrient-deprived, and low-pH environments, conditions that are specifically found in malignant tumours. The combination of radiotherapy with hyperthermia increases cytotoxic effects. Several clinical randomised trials in different tumour sites have shown benefits from combined treatment (Zee et al., 2000).

When any tissue is heated, various physiological changes occur, the majority of which are secondary to changes in blood flow (Song, 1978; Vaupel and Kallinowski, 1987; Horsman, 2006). Blood flow is also one of the major vehicles by which heat is dissipated from tissues; thus, the tissue blood supply will have a significant influence on the ability to heat tissues (Patterson and Strang, 1979). The lower the rate of blood flow, the easier it is to heat. Although solid tumors can have blood flow values that can be greater than that of certain normal tissues, when compared to normal tissues the tumor blood supply is generally primitive
and chaotic in nature, which can result in areas that are nutrient deprived, low in oxygen, and highly acidic, and cells that exist in these adverse conditions are generally more sensitive to the cytotoxic effect of heat (Horsman, 2006).

**Hyperthermia combination with gene therapy**

Hyperthermia therapy has been applied to many advanced malignancies, but tumor regression was restricted to thermosensitive types of cancer. To elucidate ways to overcome thermoresistance and improve the therapeutic efficiency of treating thermoresistant cancer by gene therapy researchers focused on the expression of heat shock protein 70 (HSP) (Ohtsuru et al., 2001).

The heat shock protein 70 family is a family of multifunctional repair/removal agents for denatured and damaged proteins that can enhance cell survival following injury caused by many different agents including heat, radiation, and chemotherapeutic agents. Basal level cellular expression of inducible HSPB1 is upregulated in many cancer cells and confers a high level of resistance to radiation and chemotherapeutic agents in the absence of heat (Kaur et al., 2011).

A long line of experimental evidence positions Hsp70-1 as a cancer relevant survival protein. It is abundantly expressed in malignant tumors of various origins (Jaättele, 1999; Mosser and Morimoto, 2004), and its expression correlates with increased cell proliferation, poor differentiation, lymph node metastases and poor therapeutic outcome in human breast cancer (Ciocca et al., 1993; Vargas-Roig, 1998).

In hyperthermia, HSPs are thought to be involved in the protection of cells against heat damage (Hildebrandt et al., 2002).

As discussed above, due to HSP family are responsible for survival and resistance of cancer cells to heat, they can be thought as target proteins in cancer treatment. Therefore inactivation of HSP genes by gene knockout technology will become one of the new approaches in the field of oncology.

**The effects of hyperthermia on angiogenesis mechanism**

Angiogenesis, the formation of new blood vessels from the endothelium of the existing vasculature, is fundamental in tumor growth, progression and metastasis (Folkman, 1990).

Cancer cells are capable of stimulating angiogenesis by producing several angiogenic factors, which include vascular endothelial-derived growth factor (VEGF), angiopoietins, basic fibroblast-like growth factor (bFGF), epidermal growth factor (EGF), interleukin 8 (IL-8), and transforming growth factor β (TGF-β) besides numerous other molecules (Eichhorn et al., 2007).

Tumor cells can penetrate blood or lymphatic vessels, circulate through the intravascular stream, and then proliferate at another site: metastasis. Tumor growth and metastasis depend on angiogenesis and lymphangiogenesis triggered by chemical signals from tumor cells in a phase of rapid growth (Folkman, 1971).

Vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor-a (TGF-a) and platelet-derived growth factor all have been identified as angiogenic factors. Among them, VEGF is considered to be the pivotal factor in tumourneovascularisation, because it increases in endothelial-cell proliferation and migration, enhancement of tumour growth in vivo and remodelling of perivascular matrices by augmenting proteinases such as matrix metalloproteinases (MMPs) (Sawaji et al., 2002).

Heat treatment of ECs (endotelial cells) strongly inhibits their differentiation into vascular structures both in Matrigel assay, which recapitulates numerous aspects of angiogenesis, including cell migration, differentiation, and metalloproteinase activation (Grant et al., 1991), and in spheroid assay, which mimics vessel sprouting from a preexisting one (Korff and Augustin, 1998).
The effects of hyperthermia on cancer stem cells

In recent years treatment of cancer have gained a new direction with the discovery of cancer stem cells. If stem cell differentiation potential becomes impaired and their proliferative capacity becomes uncontrolled, these mutated, potentially tumorigenic, self-renewable stem cells have the potential to cause cancer and are called cancer stem cells (CSCs). CSCs are responsible for tumor relapse and metastasis (Cetin and Topcu, 2012). This subpopulation of tumor mass frequently survives anticancer treatment when exposed to radiation and cytotoxic drugs and they are responsible for the failure of most, if not all, anticancer therapies (Sabisz and Składanowski, 2011). Therefore CSCs have become novel targets for treatment of cancer. In this content the effects of hyperthermia on CSCs are focused by researchers. Nanoparticle-mediated hyperthermia can also sensitize CSCs to other treatments such as ionizing radiation exposure. This type of bipartite therapeutic approach was investigated by Atkinson et al. (Atkinson et al., 2010). Multiple assays for CSCs, including side population phenotype, aldehyde dehydrogenase expression, mammosphere formation, and in vivo xenotransplantation, indicated that magnetic hyperthermia reduced or, in some cases, eliminated the CSC subpopulation in treated cells (Sadhukha et al., 2013).

In addition to CSCs which are responsible for tumor relapse, mesenchymal stem cells contribute to the formation of the tumor microenvironment. Therefore mesenchymal stem cells have become one of the important targets in cancer treatment similar to CSCs. In a study by Cho et al. the morphological analysis and cell proliferation assay showed a reduced viability of the tumor cells in the conditioned medium with the heat treated mesenchymal stem cells (Cho et al., 2009).

DISCUSSION

Although there are numerous medications, treatment methods and major advances to treat cancer, a certain percentage of cancer patients can survive. Therefore researchers are constantly entering into new searches. In recent years hyperthermia is one of the novel therapeutic approaches in oncology. Hyperthermia treatment is a multi-disciplinary method based on medical and technical aspects. It involves various professional groups, including radiation oncologists, medical oncologists and medical physicists, as well as engineers and technical personnel (Bruggmoser et al., 2012). Although it has known as a method in treatment of variety of diseases for centuries, in these days it has been used alone or combination with conventional treatment methods such as surgery, chemotherapy and radiotherapy in oncology field. Chemotherapy and radiotherapy are used in clinic routinely, but doses are administered may not be sufficient to cure the disease all the time. So effectiveness of these treatments are tried to increase with various methods. Various trials showed that hyperthermia enhances the efficacy of various chemotherapeutic drugs and radiation. However, implementation of the three treatment modalities (chemotherapy, radiotherapy and hyperthermia) with different combinations bring about changes in treatment activities. The combination of gimeracil which inhibits HR in the DNA DSBs repair mechanism and hyperthermia (42°C, 43°C, or 44°C) sensitized radiation more effectively than each modality alone since gimeracil sensitized effects of both radiation and hyperthermia. (Takagi et al., 2012). Re-irradiation plus hyperthermia is an effective treatment for recurrent breast cancer with acceptable toxicity. Results from five randomized trials have shown that the complete response rate for breast cancer recurrences increases from 41 to 59% when hyperthermia is combined with radiotherapy (Linthorst et al., 2013).

In the context of gene therapy heat shock protein family play an important role. Because of heat shock protein are thought to be involved in the protection of cells against heat damage (Hildebrandt et al., 2002), their knockout is an issue to be dealt with. Prevention of the mechanisms that contribute to tumor growth is one of the goals of oncology. Angiogenesis is an important mechanism for tumor growth. Therefore researches focus on prevention of these mechanisms...
effectively. Inactivation of various molecules involved in angiogenesis with hyperthermia is an important therapeutic approach in order to prevention of tumor growth.

In recent years targeted therapies have begun to change places with conventional therapies. In this respect cancer stem cells are very important. These cells are responsible for the tumor recurrence and their elimination is very difficult with conventional methods. Therefore destroying of these cells with hyperthermia that increases the effectiveness of standard treatments can become a novel therapeutic approach.

In conclusion we believe that hyperthermia which began with retrospective preclinical studies have come into question with development of new application methods currently will contrubute to cancer treatment.

REFERENCE


