- Review -

Effects of Regional Hyperthermia with Moderate Temperature on Cancer Treatment

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Despite that moderate hyperthermia can exert various antitumor activities such as direct cytotoxic effects on tumor cells, effects on tumor vasculatures and immunological effects, hyperthermia has been usually combined with radiotherapy or chemotherapy due to its limited efficacy in cancer treatment, showing some positive clinical benefits with generally well-tolerated side effects. Since heat shock responses itself can interfere with the anti-tumor effects of hyperthermia, not all of these studies might have demonstrated positive clinical outcomes in cancer patients. Therefore, the negative anti-tumor effect of hyperthermia should be reduced to enhance the effectiveness of hyperthermia. Although the responses to heat stress of tumor tissues containing vessels, immune cells, connective tissues as well as cancer cells, are very complicated, it is needed to study in the near future if some clinically available drugs, which can modulate heat stress responses, can improve the efficacy of hyperthermia in patients with cancer. In this review, the effect of clinical hyperthermia centered on non-invasive external hyperthermia using radiofrequency at moderate temperature will be discussed, since it is the state-of-the-art technology in the current clinical practice of hyperthermia, and a moderate operational temperature is used to increase the therapeutic effectiveness of conventional therapy without additional toxicity to normal tissues.

Key words : Cancer, Hyperthermia, Heat stress, Heat shock response

Introduction

Currently cancer is one of the leading causes of human death worldwide, despite that the three major cancer treatments including surgery, chemotherapy and radiation therapy are being continuously improved. To increase the survival of cancer patients, combination of other therapeutic modalities to current therapies may be required. Many anticancer therapies are under development; for example, immunotherapy, hyperthermia, photodynamic therapy and gene therapy etc. Among these, hyperthermia is one of promising anticancer therapy, since it has been demonstrated that hyperthermia can be used as an adjuvant therapy for the standard cancer treatments such as radiotherapy and chemotherapy [72, 77].

The fundamental idea of heat on cancer was originated from the ancient period, during which Hippocrates was aware of the potential of heat to reduce or eliminate tumors [1], and after that Busch reported complete remission of sarcoma after a high fever due to erysipelas infection in 1868 [28]. This led to development of Coley toxin, named after William B. Coley who had experienced complete responses in 60 out of 210 patients with soft tissue sarcoma for at least 10 years [67]. Although Coley had repeatedly stated that the higher and the longer the fever, the better the effect of the treatment [55], the major effects of Coley's Toxin might come primarily from immunological effects as well as thermal effect [28]. After Coley's death in 1936, clinical interests in the use of his vaccine diminished in preference to the more broadly applicable radiation therapy and chemotherapy [67].

However, almost at the same time with Coley, in 1898 Westermark observed the pure thermal effects on various gynecological diseases using long-term (48 hr) local (by virtue of intravaginal metal coil heated with circulated water to 42~44°C) and regional (hot tubs) heating, showing the effect of the long-term hyperthermia to treat cancers without damages to healthy normal tissues, and the concept of elec-

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tromagnetic therapy started from works of Nicola Tesla and Arsen d'Arsonval [55]. Currently, a variety of clinical hyperthermic technologies including thermal conduction using a circulating liquid and exposure by electromagnetic (radiofrequency, microwaves or infrared) or acoustic waves (ultrasound) have been developed and can be broadly categorized into local, regional or whole-body hyperthermia [16, 23].

In this review, the effect of clinical hyperthermia centered on non-invasive external hyperthermia using radiofrequency at moderate temperature (41~43°C) will be discussed, since it is the state-of-the-art technology in the current clinical practice of hyperthermia, and an operational range of moderate temperature is used to increase the therapeutic effectiveness of conventional therapy without additional toxicity to normal tissues [18, 29].

Mechanisms of antitumor effects of hyperthermia

The effects of hyperthermia on tumor would be direct or indirect due to its multifactorial effects, and be dependent on temperature and exposure time. Basically, the higher the temperature and exposure time, the higher the direct cytotoxicity of hyperthermia. However, a high temperature and exposure time can injure normal tissues and hamper the indirect effects of hyperthermia on vasculature and immune cells.

Direct cytotoxic effects of hyperthermia

When exponentially growing cells were exposed to a predefined temperature between 41 and 47°C in vitro, two phases of direct cytotoxicity were observed in a dose-survival curve, which showed a first linear growth arrest in the beginning of heat exposure, which represents a reversible, non-lethal heat damage, and a second exponential cell death reflecting an irreversible cytotoxicity [17, 27, 52]. In this study, temperature of 43°C seemed to be a critical breakpoint to induce significant cell death, since the induction of cell death at lower temperatures below 42~43°C is remarkably lower than that at higher temperature above 43°C. This result is related with the reference temperature of 43°C for calculation of thermal dose, CEM 43°C T90, the number of cumulative equivalent minutes at 43°C exceeded by 90% of monitored points within the tumor. A thermal dose (D) derived from exposure time (t) and given temperature (T) can be calculated as $D = tR^{(43-T)}$ with R=0.25 for temperatures ≤43°C, and R=0.5 for temperatures > 43°C, and 10 CEM

43°C T90 is usually considered as the goal of the hyperthermic therapy [17, 57], since it is difficult to uniformly increase the temperature of human tumors above 43°C *in vivo* without damages to normal tissues using the currently available hyperthermia devices.

An increased temperature results in both unbalanced metabolism and changes in cellular structures such as membranes and macromolecules [27, 41]. The molecular effects of hyperthermia are dysfunction of cell membrane including changes in fluidity of cell membrane and impairment of membrane transport, protein denaturation, impairment of DNA/RNA/protein synthesis, inhibition of DNA repair enzymes, and alterations of intracellular metabolism, gene expression and signal transduction [41, 54]. These cellular responses to hyperthermia are thought to be rerated with the cytotoxic effects of hyperthermia, and protein denaturation seemed to be the rate limiting step for hyperthermic cell death and for any other thermal effect with a high activation energy, since the thermal energy dose required for cellular protein denaturation is closely correlated to that required to induce exponential cell death [27, 41].

Although many preclinical and clinical studies have shown that cancer cells are more sensitive to moderate hyperthermia (42~45°C) than normal cells, it is unknown why cancer cells exhibit this distinctive susceptibility to moderate hyperthermia at molecular and cellular levels. Recently, a genomics approach involving microarray, bioinformatics, and network analysis of the global transcription changes revealed that hyperthermia specifically disrupts the expression of key mitotic regulators including *KIF11*, *CDK6*, *STAG2*, *NEK2*, *CHUK*, *KPNA4*, *CENPF*, and *NCAPG*, and G2/M phase progression in the breast cancer cells, compared with mammary epithelial cells, suggesting that the selective disadvantage of breast cancer cell lines in response to hyperthermia may be due to an inability to correctly regulate their core biological processes and mitotic cell cycle machinery [2].

Effects of hyperthermia on tumor vasculature

Besides direct cytotoxic effect of hyperthermia, high temperature has indirect effects on tumor growth through change of intratumoral blood flow *in vivo*. The tumor vasculature is very different from that of normal tissues, since angiogenesis in tumor is unable to keep pace with the rapid proliferation of neoplastic cells. Due to structurally chaotic vasculatures in tumors, tumor vessels have numerous func-

tional abnormalities, such as unstable speed and direction of blood flow, high vascular resistance, and increased vascular fragility, and consequently abnormal areas develop within the tumor that are characterized by deprivation of glucose and energy, high lactate levels, extracellular acidosis, and oxygen deficiency [64].

The change of intratumoral blood flow caused by heating is dependent on the temperature and duration of hyperthermia. It has been demonstrated that in FSaII murine fibrosarcoma model the pO_2 increased progressively during 1 hr heating at 41.5°C, and heating at higher temperatures failed to increase or even reduced the pO_2, and in R3230 rat mammary adenocarcinoma model the highest level of pO_2 was achieved by heating at 42.5°C for 30 min [33], suggesting that modest temperature hyperthermia may be an efficient and useful means to improve the effect of radiotherapy and chemotherapy through an increased oxygenation and drug delivery in human tumors. However, at higher temperature and longer duration, tumor tissue could be overheated. In normal tissue blood vessels dilate in response to high temperature, resulting in an increase in blood flow through the heated region and a consequent dissipation of heat. In contrast, aberrant vessels in tumor tissues are not able to dilate, some of them collapse, and consequently intratumoural temperature will increase due to loss of heat dissipation. [11]. In addition, delivery of oxygen and nutrient to heated tumor is impaired, and intratumoral lactic acidosis is induced [27]. Finally, the heated tumor cells will undergo necrotic or apoptotic death.

Immunological effects of hyperthermia

In addition to local effects on tumor tissues of hyperthermia described above, hyperthermia can work systemically through stimulation of tumor-specific immunity, and consequently can be regarded as in situ anti-tumor vaccine. The heat shock response, a major response to stress conditions in the cytosol [47], is mediated by heat shock factor 1 (HSF1), a transcription factor [80]. In unstressed cells HSF1 is sequestered as a monomer in the cytoplasm by Hsp90 and co-chaperones. When cells are heated, accumulation of denatured and misfolded cellular proteins results in depletion of chaperones available for the assembly of HSF1 inhibitory complex, and upon its release from the Hsp90 complex an active HSF1 trimer is formed and subsequently enhances the transcription of the so-called heat shock genes [61, 68, 73].

The heat shock proteins (Hsps) including Hsp70, Hsp90 and gp96, an endoplasmic reticulum paralog of Hsp90, can form complexes with a broad spectrum of cellular proteins and peptides due to their chaperone functions. In addition to their intracellular chaperoning functions, extracellular complexes of Hsps play key roles in eliciting antitumor immune responses. Vaccination with these complexes elicits specific immunity against the tumor from which the Hsps were purified, albeit the immunogenicity of Hsp90 was approximately 10% of that of gp96 or Hsp70 [6, 26, 32, 63, 66, 70]. It has been known that Hsp70 either alone, bound to exosomes or in combination with tumor-derived peptides, which can be released from heat-stressed tumor cells, is able to stimulate the maturation and antigen-presenting function of dendritic cells (DCs), activate NK cells and T cell, and induce the release of pro- and anti-inflammatory cytokines from macrophages [20, 63, 65, 69]. In addition, following non-lethal heat stress Hsp72 can be expressed on the cell surface of heat-stressed tumor cells including sarcoma and leukemic cells, and subsequently these cells can be recognized and killed by NK effector cells through a heat-inducible immunogenic determinant associated with Hsp72 [49, 50, 48]. The effects of moderate temperature may be similar with those of radiofrequency ablation in the transitional zone in which various inflammatory cells such as neutrophils, macrophages, DCs, NK cells, as well as B and T cells are infiltrated by stimulation with various immunogenic intracellular substrates including RNA, DNA, Hsps, uric acid and high mobility group protein B1 (HMGB1) released from the sublethally heat-damaged cells [12]. Recently, spontaneous regressions of multiple distant metastatic lesions have been reported after percutaneous radiofrequency ablation of primary or recurrent renal cell carcinoma, suggesting a possible mediation of systemic antitumor immune response induced by local hyperthermia [36, 56]

In addition to Hsps, some of NKG2D (natural-killer group 2, member D) ligands with heat shock element in their promoter that bind HSF1, can be induced by heat shock [22, 37]. NKG2D ligands can be recognized by NKG2D, one of the most important activating receptors expressed on the vast majority of NK and NKT cells, CD8+ T cells and γδ T cells, and on certain subsets of human CD4+ T cells [42, 53]. Therefore, hyperthermia may increase tumor cell recognition by immune cells with NKG2D receptor via induction of NKG2D ligands on heat-treated tumor cells.

Self-interference of hyperthermia with its anti-tumor effects

Despite of various antitumor effects of hyperthermia, the clinical results obtained by hyperthermia alone have not been satisfactory. Due to the limited efficacy of hyperthermia in cancer treatment, hyperthermia has been usually combined with radiotherapy or chemotherapy, showing positive clinical benefits with generally well-tolerated side effects in randomized trials with a variety of malignancies, possibly through the effects of hyperthermia described above [13, 16, 43]. Although many of these studies have demonstrated positive clinical outcomes when hyperthermia is combined with other treatments, but not all, possibly due to some technical problems, such as the inability of real-time non-invasive monitoring of tissue temperature, and difficulties to focus heat to the target tumors. In addition to these technical problems, heat shock responses itself may interfere with the anti-tumor effects of hyperthermia, playing as double-edged swords.

Although Hsps can be induced as a major heat shock response and stimulate tumor-specific immunity, the induced Hsp27, Hsp70, and Hsp90 can inhibit both caspase-dependent and -independent apoptotic pathways at various levels, providing protection of cells from apoptosis-inducing stimuli such as radiotherapy, chemotherapy and second heat shock [7, 8, 31, 35, 40]. Therefore, inhibition of Hsps function may be required to improve efficacy of hyperthermia.

Tumor-cell derived exosomes are considered as an efficient anti-tumor vaccine, since they are a source of tumor antigens [3, 76]. Exosomes harvested from various heated human tumor cells carry enriched tumor antigens and chemokines, such as CCL2, CCL3, CCL4, CCL5 and CCL20, and act as an efficient anti-tumor vaccine [10, 15, 79]. However, tumor-derived exosomes have also immunosuppressive properties [44]. It has been shown that thermal and oxidative stresses enhance the release of immunosuppressive exosomes bearing NKG2D ligands in both Jurkat leukemia T cells and Raji lymphoma B cells [25]. Exosomes can express differentially and constitutively NKG2D ligands from both MICA/B (MHC class I-related chain A/B) and ULBP (UL16-binding proteins) families on their surface. Consequently, the NKG2D ligand-expressing exosomes serve as decoys with a powerful ability to downregulate the NKG2D receptor and impair the cytotoxic function of NK and NKT cells, CD8+ T cells, γδ T cells and CD4+ T cells [39, 46]. Some tumor cells release proapoptotic exosomes bearing death ligands such as Fas ligand and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), inducing apoptosis of activated T cells [4, 30]. Exosomes can also enhance the immunosuppressive functions of myeloid-derived suppressor cells (MDSCs) and regulatory T cells through a TGF-β-dependent mechanism [14, 69, 71]. Recently, it has been shown that an interaction between tumor-derived exosomes-associated Hsp72 and MDSCs mediates the immunosuppressive activity of the MDSCs via activation of STAT3 in a TLR2/MyD88-dependent manner through autocrine production of IL-6 [9].

Meanwhile, hyperthermia can suppress the function of immune cells directly. Several studies have demonstrated impairments of cytotoxic function in lymphocytes, especially NK cells, after heat treatment. Cytotoxic activities of NK cells and lymphokine-activated killer cells could be enhanced at febrile range ($\leq 40^{\circ}$C), but were significantly reduced at 42°C for 1 hr [62, 78]. When the effector and target cells were exposed simultaneously to hyperthermia (42°C for 1 hr) during the cytotoxicity assay, the lytic activity of NK and IL-2-activated NK cells was remarkably reduced [21]. The impairment of human NK cell cytotoxicity may result from downregulation of perforin, and is transient in vitro and in vivo [20, 24]. Therefore, due to these non-beneficial effects of hyperthermia on immune cells, temperature about 42°C may be appropriate for locoregional hyperthermia.

Tuning of hyperthermia

As described above, various mechanisms which are activated by hyperthermia are involved in interference of the antitumor effects of hyperthermia. To enhance the effectiveness of hyperthermia, the negative anti-tumor effect of hyperthermia should be reduced. Basically, it is not possible to remove only the negative side of hyperthermia in the treatment of cancer, while keeping the positive effect of hyperthermia. Nevertheless, we need to find out the best way to enhance the antitumor effects of hyperthermia.

The cytoprotective functions of Hsps are essential for survival of cancer cells. Therefore, inhibition of Hsps may be able to improve direct cytotoxic effects of hyperthermia. Hsp90 has regarded as a major pharmaceutical target in cancer therapy, due to its function of molecular chaperone, which is known to bind and stabilize numerous oncoproteins with activity in the cell cycle, signal transduction and transcription [5, 74]. However, inhibition of Hsp90 results in disruption of complexes of Hsp90 with HSF1, thereby

causing HSF1-mediated induction of cytoprotective Hsp70 and Hsp27, which contribute to resistance to Hsp90 inhibitors [51]. Therefore, inhibition of Hsps or HSF1 would be required to prevent the Hsp90 inhibitor-mediated rebound induction of cytoprotective Hsps. In addition, Hsp70 neutralization induces tumor regression in animal models of colon cancer and melanoma by inducing anti-tumor immune responses [60], and this antitumor immunity might be explained by the prevention of the interaction between tumor-derived exosomes-associated Hsp72 and MDSCs [9]. Currently, many inhibitors of Hsps are under development and clinical trials [34]. HSF1 may be also a good target to enhance the efficacy of hyperthermia, since HSF-1 is a key regulator of Hsp90 and Hsp70 expression. Several small molecules such as quercetin, KNK437 and triptolide are identified as inhibitors of HSF1 and entered into clinical development [75]. However, development of specific inhibitors of Hsps and HSF1 has been delayed, and currently are not available commercially. Recently, it has been reported that ibuprofen, a nonsteroidal anti-inflammatory drug, significantly suppresses Hsp70 expression by depleting HSF1 in lung adenocarcinoma-derived A549 cells [19]. In this study, ibuprofen enhances the anticancer activity of cisplatin, possibly through suppression of Hsp70 expression. Therefore, it is needed to study if ibuprofen can improve the efficacy of hyperthermia in cancer patients.

Like Hsps, tumor-derived exosomes also have pro-tumorigenic as well as anti-tumorigenic properties. Therefore, depletion of tumor-derived exosomes may give some benefits to survival of cancer patients. It has been demonstrated that dimethyl amiloride, an anti-hypertensive drug that also inhibits exosome formation [58], can decrease exosome production and consequently reduces suppressor functions of MDSCs from cancer patients, restoring the efficacy of low dose cyclophosphamide, which is related to its capability of inducing a T cell-dependent immune response through elimination of regulatory T cells rather than to its cytotoxic effect on tumor cells [9]. Another promising strategy to remove exosomes from the entire circulatory system involves extracorporeal hemofiltration system [44]. If the exosome from heat-stressed cancer cells can be decreased, the efficacy of hyperthermia would be increased through an enhanced anti-tumor immunity.

Although a variety of anti-tumor vaccines is currently being evaluated, only one therapeutic cancer vaccine (sipuleucel-T) has been approved by the US Food and Drug
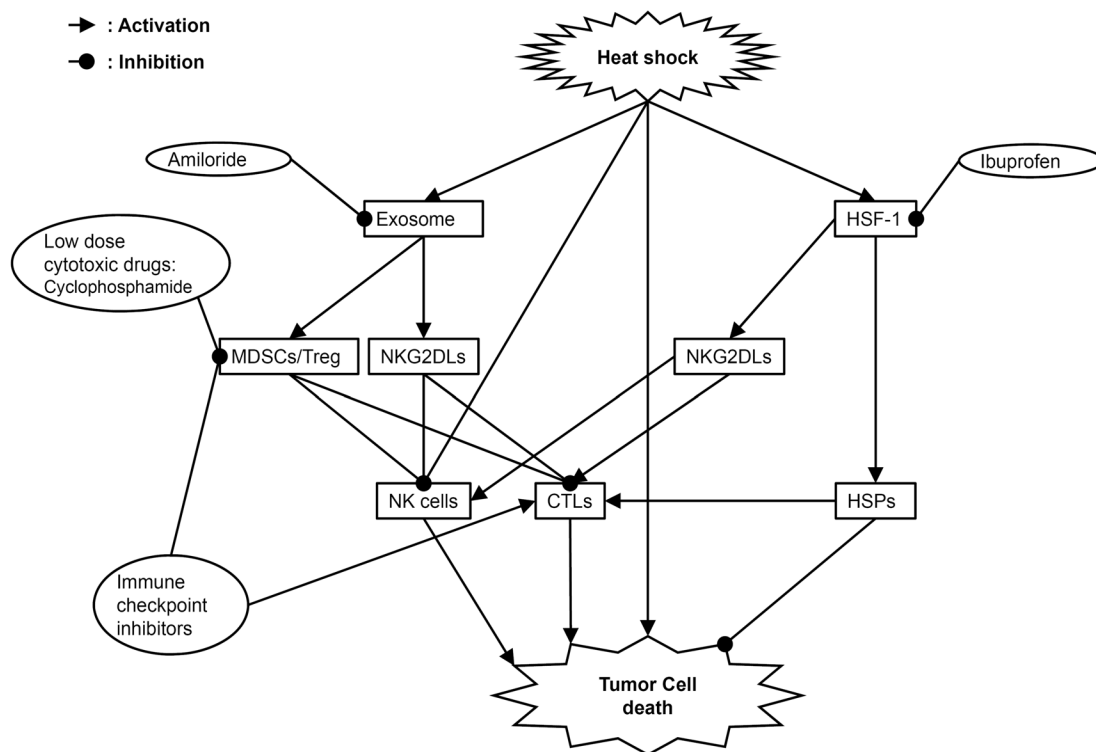

Fig. 1. Anti-tumorigenic and pro-tumorigenic effects of hyperthermia and modulation of heat shock responses with clinically available drugs (ellipses) to improve the anti-tumorigenic effects of hypethermia.

Administration for the treatment of cancer, possibly due to profound influence of immunosuppressive microenvironment of tumor, limiting the effectiveness of anti-tumor vaccines [59]. The efficacy of hyperthermia as an in situ anti-tumor vaccine may also be limited by same causes. Currently, numerous strategies including the combination of vaccines with immune checkpoint inhibitors, certain chemotherapeutics, small-molecule targeted therapies, and radiation are being evaluated to counteract the immunosuppressive microenvironment of tumor [59]. Among those the immune checkpoint inhibitors including anti-CTLA4 and anti-PD1 monoclonal antibodies are at the center of current development of combination with vaccines [45, 59], and many cytotoxic anticancer drugs given in lower-than therapeutic doses can not only eliminate tumor cells but also block the immunosuppressive activities in tumor microenvironments and consequently favor the development of anticancer immune responses [38]. Therefore, clinically available cytotoxic drugs in low doses and the immune checkpoint inhibitors can be considered for their early combination with hyperthermia to enhance its efficacy.

Although the responses to heat stress of tumor tissues containing vessels, immune cells as well as cancer cells, are very complicated, and it is hard to expect the consequences of treatment with modulators of heat shock responses, it is needed to study in the near future if some clinically available drugs such as ibuprofen, amiloride, immune checkpoint inhibitors, and low dose of some cytotoxic anticancer drugs, which can modulate heat stress responses, can improve the efficacy of hyperthermia in cancer patients (Fig. 1).

Acknowledgment

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초록 : 국부 중등도 온열요법의 암치료 효과

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중등도 온열요법이 종양세포에 대한 세포독성, 종양혈관에 미치는 영향 및 면역학적 영향 등 다양한 항종양 활성을 가지고 있음에도 불구하고, 중등도 온열요법은 그 자체만으로는 항암효과가 뚜렷하지 않아, 방사선치료나 항암제 치료와 병용하여 암치료에 사용되고 있으면서, 심각한 부작용이 없이 어느 정도의 긍정적인 효과를 보이고 있다. 모든 연구에서 긍정적인 결과를 보이지 못한 것은 열충격 반응 그 자체가 온열요법의 항암효과를 방해하기 때문이다. 그러므로 온열요법의 효과를 증가시키기 위해서는 온열요법의 항암효과에 대한 부정적인 영향을 제거해야 한다. 암세포뿐만 아니라 혈관, 면역 세포 및 결체조직 등을 포함하고 있는 종양조직의 열 스트레스에 대한 반응은 매우 복잡하지만, 임상적으로 사용되고 있는 약물 중 열 스트레스 반응을 조절할 수 있는 약물들이 암환자의 온열요법 치료 효과를 개선시킬 수 있는 지에 대한 연구가 필요하다. 이 종설에서는 현재 임상에서 사용하고 있는 온열요법 장치로서 최신의 기술이며, 중등도 온도가 정상 조직에 대한 부작용 없이 기존 치료법의 효과를 증가시킬 수 있기 때문에, 비침습적 체외용 고주파 중등도 온열요법을 중심으로 다룬다.