Review

Tumor Targeting with Hyperthermia

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Abstract: Is it possible to produce the specific death of tumor tissue using hyperthermia? An answer requires an understanding of the temperatures used. Hyperthermia at 43°C or above causes acute vascular disturbance and protein degeneration in all tissues, leading to the necrosis of tumors. Radiofrequency ablation (RFA) is a tumor-specific physiological targeting method for small tumors, and hence, biology may not greatly contribute to the progress of such a high-temperature therapy. In selective targeting of tumors with mild-temperature hyperthermia (MTH), two separate issues require consideration: the effect on the tumor cells themselves and the effect on the tumor environment. In targeting tumor cells, optimal MTH efficacy is dependent on the inhibition of thermotolerance. The availability of a drug that selectively inhibits the expression of hsp could render MTH an effective form of anti-tumor therapy. At present, however, because MTH itself has only a weak effect on tumors, some method of concomitant therapy is necessary. Studies involving concomitant treatment of MTH with low-dose-rate (LDR) irradiation or pulsed-dose-rate (PDR) irradiation have shown that the combination of MTH with irradiation allows the inhibition of tumor recovery from sublethal damage inflicted by a rise in temperature of just 1 or 2°C. Because MTH induces not only an increase of the expression of tumor-specific antigens, but also an increase in the uptake of antibodies over an extended period by altering hemodynamics, recommended clinical use is in combination with radioactive monoclonal antibody therapy. MTH has also gained attention in the field of gene therapy. The activation of hsp by heat produces a dynamic response, and attempts are now underway to promote the efficient expression of a cytotoxic cytokine gene within tumors using an hsp 70 promoter. With regard to targeting of the tumor environment, the effect of MTH on tumor blood vessels is important. MTH has been shown to improve intratumoral hypoxia, while more recent drug-delivery research has investigated the use of MTH in the selective uptake of antitumor drugs. This review will outline these new research results and address the question of how hyperthermia can be used effectively in tumor therapy.

Key Words: cancer, hyperthermia, mild temperature, targeting

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1. Introduction

Hyperthermia is a treatment which targets cancer cells directly or targets the environment surrounding the tumor cells (Table I). In the biology of classic hyperthermia, it is known that a change is effected in the inactivation energy of cells at temperatures above 43°C. Many previous early-stage clinical studies failed because they attempted to achieve sustained high-temperature heating of tumors (10). Heating technology has recently advanced, and many clinical trials have demonstrated advantages of clinical use of hyperthermia (3–6). The occurrence of a variety of unknown phenomena has been observed in the MTH. This review will outline new research results and consider how hyperthermia can be used effectively in clinical care.

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<th>Table I. Tumor targeting using hyperthermia</th>
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<td>① targeting tumor cells</td>
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<td>2) microwave probe</td>
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<td>2: mild temperature hyperthermia (around 41°C)</td>
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2. High-temperature Hyperthermia

Temperatures of 43°C and higher not only have a destructive effect on cancer cells themselves, they also cause necrosis of the entire tumor structure through the blockage of tumor blood vessels. One method of selective heat delivery is radiofrequency ablation (RFA) (39). RFA involves the insertion of electrodes into tumors to apply heat to the tumors. The rate of tumor suppression is extremely high, but it is difficult to apply this method to tumors of 3 cm or more. Other effective methods are microwave surgery (9) and photocoagulation (10). Selective thermal delivery to tumor tissue recently involves the use of magnetic fields (11–13). Hyperthermia using magnetic fields requires the administration of a magnetic substance as selectively as possible to the tumor. A feature of this form of treatment is that a rapid increase in temperature can be achieved in the target tissue, whilst other tissues that do not take up the magnetic substance are entirely unaffected (41). Both of these methods involve selective high-temperature thermal delivery to tumors, and may be considered to be forms of targeting based on physical engineering.
3. Mild-temperature Hyperthermia

3.1. Thermotolerance

MTH at 40 or 41°C, if used alone, has almost no cytotoxic effect. It is known that under conditions of continuous heat elevation, cells develop chronic thermotolerance. If this thermotolerance can be inhibited, however, the cytotoxic effect of the heat would increase. Drugs reported to inhibit thermotolerance are shown in Table II\(^{13}\). KNK437 (N-formyl-3,4-methylenedioxy-benzylidene-\(\gamma\)-butyrolactam), a benzylidene lactam compound, has been reported to inhibit the development of thermotolerance\(^ {16}\). This compound has been shown to be more effective than quercetin in attenuating thermotolerance and in inhibiting various hsps at the mRNA level. Koishi et al reported that the simultaneous use of KNK437 with hyperthermia in vivo significantly increased the cytotoxic effects of this drug via the inhibition of hsp72\(^ {17}\).

<table>
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<th>Table II. Targeting HSP70-induced thermotolerance for design of thermal sensitizers</th>
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<td>Bioflavonoid drugs</td>
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<tr>
<td>quercetin (3, 3', 4', 5-7-pentahydroxyflavone)</td>
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<tr>
<td>luteolin</td>
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<tr>
<td>apigenin</td>
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<tr>
<td>genistein</td>
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<tr>
<td>Non-steroidal anti-inflammatory drugs (NSAIDs)</td>
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<tr>
<td>sulindac</td>
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<tr>
<td>indomethacin</td>
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<tr>
<td>ibuprofen</td>
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<tr>
<td>aspirin</td>
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<tr>
<td>sodium salicylate (NaSal)</td>
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<tr>
<td>Anti-sense oligonucleotides against HSP70 mRNA</td>
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<td>Drug-screen candidates</td>
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Our research using the thermotolerance inhibitor KNK437 in combination with mild hyperthermia at 41°C showed the inhibition of heat shock protein (hsp) 70 expression and clear cell death\(^ {18}\) (Fig. 1). If a drug such as KNK437 can be selectively delivered to tumors, a significant increase in the effect of hyperthermia can be expected.

3.2. Combined with Radiation Therapy

There are two types of radiation therapy: high-dose-rate irradiation (HDRI) and low-dose-rate irradiation (LDRI). LDRI is widely used to treat head and neck cancer and gynecological tumors as brachytherapy. Cell recovery occurs during LDRI therapy, however, but can be inhibited by mild hyperthermia used concurrently, which produces a sensitizing effect with only a very slight elevation of temperature\(^ {19}\) (Fig. 2). In clinical settings, brachytherapy is used to selectively irradiate tumors, so that hyperthermia sensitization occurs only in the targeted tumors\(^ {20}\).
3.3. Radioimmunotherapy

This approach of using LDRI against cancer cells has also been adopted in the combination of MTH and radioimmunotherapy\textsuperscript{21,22}. In this method, tumor-specific antibodies are labeled with radioactive isotopes and injected into the body. The administered tumor-specific antibodies bind to the tumor, where they are exposed to LDRI. This form of therapy has the following advantages: 1) through concurrent use of MTH and LDRI, repair of sublethal damage is inhibited, 2) the uptake of antibodies is increased, and 3) there is an increase in cancer-specific antigen presentation on the cell surface. Figure 3 shows the results of flow cytometry measurement of the amount of carcinoembryonic antigen (CEA) after hyperthermia treatment of human cancer cells producing CEA\textsuperscript{23}. Hyperthermia at 41°C clearly increased the amount of CEA on the cell surface. Because the increase in antibody delivery to cancer cells through hyperthermia lasts for only about 1 or 2 days, after which antibody levels return to those in normal tissues, the use of nuclides with a short half-life is considered appropriate for this kind of therapy\textsuperscript{24}.

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure1.png}
\caption{Survival curves of A549 cells in combination of KNK437 and mild temperature hyperthermia at 41°C. Development of Hsp 70 in 41°C alone and 41°C with KNK437 is also shown (reference 18).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Response of LK87 cells to mild hyperthermia plus low dose-rate irradiation (50 cGy/h) (reference 19).}
\end{figure}
3.4. Gene Therapy

It is known that hsp reacts dynamically to changes in temperature. This phenomenon is also satisfactorily achieved with MTH. Research into inducing an increase in the expression of therapeutic genes within cells using hsp promoters is now underway. An example is the incorporation of enzyme genes, which activate otherwise non-active prodrugs\textsuperscript{29}, or so-called "suicide genes." Results have shown that MTH is more effective in promoting gene expression than high-temperature therapy\textsuperscript{29}. In laboratory study, further, gene therapy in combination with hyperthermia has been reported effective in suppressing the proliferation of tumor cells.

3.5. Intratumoral Oxygen Pressure

Maintenance of the internal temperature of a tumor at 43°C or higher not only kills tumor cells but also causes widespread necrosis throughout the tumor structure via damage to tumor blood vessels. Concern has been expressed that when irradiation and hyperthermia are used concomitantly, the necrosis brought about by the hyperthermia may cause an increase in hypoxic cells, which could make the radiation therapy ineffective. In the case of MTH, increased blood flow to normal tissues occurs in the early stage of treatment. Song et al.'s. investigation of hyperthermia and oxygen pressure has shown that MTH actually causes a sustained increase in the level of intratumoral oxygen\textsuperscript{37,290}. The findings have verified that one mechanism of the sensitizing effect of hyperthermia and radiation therapy is an increase in intratumoral pO₂.

3.6. Drug Delivery Systems

Heat-sensitive liposomes are used to envelop a drug substance and then release it when the
temperature rises. Those that release a drug at a temperature of around 40°C have recently been designed. MTH not only promotes the release of drugs, it also enables the targeting of tumors by increasing blood flow to tumors and accelerating vascular permeability. Extravasation of liposomes occurs from a temperature of 40°C\(^\circ\)C\(^{30}\). Research has shown that this extravasation is specific to tumor blood vessels and does not occur in normal blood vessels\(^{30}\). This drug delivery system can be said to be a tumor-specific therapy that targets the tumor environment. A system that uses thermosensitive peptides as drug carriers that aggregate at a certain temperature has also been reported\(^{31}\).

4. Closing Comments

Other future areas of hyperthermia research include use with molecular-targeted drugs and induction of antitumoral immunity. Although research into the induction of direct damage to cancers by hyperthermia is of importance, it is essential that new methods of cancer therapy be devised using mild hyperthermia as a second weapon against cancer.

Acknowledgment

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References


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ハイパーサーミアによる腫瘍のターゲッティング

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要旨：温熱療法 (hyperthermia) を利用して、腫瘍組織に選択的な死をもたらすこととは可能であろうか？このテーマに答えるには、治療をおこなう温度について考えなければならない。43 度以上の高温度のハイパーサーミアでは、あらゆる組織に血管障害とタンパク変性が急激におこり、その結果、腫瘍は壊死におちいる。RFA は小さな腫瘍に対する物理的な腫瘍特定的治療 (tumor specific treatment) である。このような高温度の治療では、おそらく生物学的寄与は乏しいであろう。マイルドハイパーサーミア (mild temperature hyperthermia; MTH) による腫瘍のターゲティングについては、熱の作用を腫瘍細胞に対する作用と腫瘍環境に対する作用とに分けて考えねばならない。腫瘍細胞をターゲットとして考えた場合、MTH それ自体の効果を増強するには、熱耐性 (thermotolerance) の抑制が必要である。もし Hsp を特異的に抑制する薬剤が開発されれば、MTH 単独でも有効な治療ができる。しかし、一般に MTH は、それ自体の腫瘍への効果が乏しいため、効果的な他の併用療法が必要である。放射線との併用の場合、わずか 1-2 の温度上昇によって亜致死障害 (sublethal damage) からの回復 (repair) が現れる。これは、低線量率組織内照射 (low dose-rate irradiation; LDRI) や PDR (pulsed dose-rate) と MTH の同時併用の研究から明らかである。MTH は、腫瘍に特異的な抗原 (tumor specific antigens) の発現を増加させるだけでなく、血流動態の変化により抗体の取り込みを長時間にわたり増加させるため、放射性モノクロナール抗体治療の併用療法として有用である。MTH は、遺伝子治療の領域でも注目されている。熱による hsp の活性化はダイナミックな反応性を有しており、hsp70 プロモーターを用いて、cytotoxic cytokine gene を腫瘍内で高発現させようとする試みがなされている。腫瘍環境をターゲットとして考えた場合、MTH の腫瘍血管への影響が重要である。以前から、MTH により腫瘍内の低酸素状態が改善されるという実験結果が報告されている。最近、MTH を利用して抗腫瘍性薬剤を腫瘍に特異的に取り込まれようとする drug delivery の研究が発展している。この review では、これらの新しい研究成果を紹介し、どのようにしてハイパーサーミアを有効に利用するかに言及したい。