Hyperthermia on skin immune system and its application in the treatment of HPV-infected skin diseases

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Abstract: In this paper, the effects of hyperthermia on cells and immune system are introduced briefly. The mechanism of action of hyperthermia on human papilloma virus (HPV)-infected skin diseases was elaborated as an example in this paper. Many studies have proved that hyperthermia affects a number of cellular and molecular constitutes in the skin immune system, involving both innate and adaptive immune responses; the efficacy of hyperthermia in treating some infectious and cancerous conditions has been validated and applied in clinics, while molecular mechanisms of hyperthermia affecting the immunereaction is still unclear.

Key words: hyperthermia; immune system; HPV-infected; immune response; tumor

1 Introduction

By definition, hyperthermia is elevated body temperature due to failed thermoregulation that occurs when a body produces or absorbs more heat than it dissipates [1]. In a broader sense, hyperthermia can also be deliberately induced using drugs or medical devices and may be systemically or locally used in the treatment of some kinds of pathological conditions. Temperature is ecologically the most important, because it is a factor that is all pervasive and in most environments lacks spatial or temporal constancy [2]. Heat-related pathologies such as heat stroke are estimated to soon become one of the most serious causes of mortality. Climatic shifts and other contemporary anthropogenic causes contribute to raise prevalence of hyperthermia incidents and associated deaths. During heat stroke, core body temperature in excess of 40 °C elicits acute tissue injury and multi-organ failure that is often fatal. The nervous system is particularly vulnerable to heat [1-3].

When exposed to altered temperatures for prolonged periods, most animals can adapt physiologically and biochemically, and this process is termed thermal acclimatization [2]. Hyperthermia does not always harm. In circumstances, fever is known to be a sign of the acute inflammatory response triggered by the body as a part of the host defense. In the practice of dermatology, local heating to a certain degree is a time-awarded method to treat certain diseases.

Skin plays a pivotal role in the regulation of body core temperature. It can work both as radiator and insulator through evaporative cooling after eccrine sweating, vaso-dilation and vaso-constriction, according to the temperature changes outside and inside the body. Excessive heating of skin may be local or systemic. In any case, physiologically, there are requirements of innervation, circulation and sweating to dissipate the heat that may cause tissue damage. The normal core body temperature of approximately 37 °C is well-conserved in vertebrates with minimal changes. This temperature is usually elevated to 39 ~ 40 °C in a febrile response to infection or other stress. In certain experimental conditions, temperatures in the range of 41 ~ 43 °C are regarded as heat shock temperatures and that above 43 °C are cytotoxic temperatures [6], although this classification remains controversial and is not widely adhered to in literatures.

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2 The effect of hyperthermia on cells

Hyperthermia has a myriad of effects on the physiology of living cells. Hyperthermia affects fluidity and stability of cellular membrane and impedes the function of transmembrane transporters and cell surface receptors. Hyperthermia also induces various changes of cytoskeletal organization (cell shape, mitotic apparatus, endoplasmic reticulum and lysosomes, etc.). Intracellular de novo synthesis and polymerization of both ribonucleic acid (RNA) and deoxyribonucleic acid (DNA)-molecules during protein synthesis are decreased in vitro at temperatures between 42 °C and 45 °C in a dose-dependent manner. Hyperthermia dose not cause severe DNA-damage itself, while it can inhibit DNA repair enzymes, such as DNA-polymerase [7]. It is experimentally shown that hyperthermia induces cell death mostly through necrotic pathway [8], while at least some cell types exhibit different susceptibilities to apoptosis induced by heat [7].

The effect of hyperthermia on cells at molecular level is complicated, which depends on the types, developmental stages and the microenvironment wherein of the cells. Moreover, different hyperthermia conditions have different impacts on cells. For example, hyperthermia induces the activation of heat shock transcription factor-1 (HSF-1), which in turn transcribes heat shock proteins (HSPs). In the case of hepatocytes, the activation of HSF-1 and the subsequent expression of gene products protect the cells from damage; while in the case of spermatocytes, the activation of HSF-1 leads to the suppression of disjunction for cells and makes the cells prone to apoptosis [9]. Prolonged mild hyperthermia could activate the HSF-1 and HSPs, as well as p38 mitogen activated protein kinase in fibroblast, thus suppressing the proliferation of fibroblasts [10].

3 The immunoregulation effect of hyperthermia

The effect of hyperthermia on immune system has attracted much attention. It has been well established that hyperthermia (as well as other stress stimuli) could induce or augment the expression of a set of highly conserved proteins, i.e., HSPs. This group of proteins function mostly as chaperones, regulators of protein folding, guides in the formation of protein complexes and regulators of protein degradation. Interestingly, some of the HSP receptors have been characterized on antigen presenting cells (APCs) (Table 1). This fact is further suggestive of an immunological role of hyperthermia in immune responses.

Table 1 Characterized HSPs and associated receptors

<table>
<thead>
<tr>
<th>HSP</th>
<th>HSP receptor(s)</th>
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</thead>
<tbody>
<tr>
<td>gp96</td>
<td>CD91, TLR-2, TLR-4, SR-A</td>
</tr>
<tr>
<td>HSP60</td>
<td>CD14, TLR-4, TLR-2</td>
</tr>
<tr>
<td>HSP70</td>
<td>CD14, CD40, CD91, LOX-1, TLR-2, TLR-4</td>
</tr>
<tr>
<td>HSP90</td>
<td>CD91</td>
</tr>
<tr>
<td>Calreticulin</td>
<td>CD91, SR-A</td>
</tr>
</tbody>
</table>

Note: TLR—toll-like receptor; CD—cluster of differentiation; SR-A—Class A scavenger receptor; LOX-1—lectin-like oxidized low-density lipoprotein receptor-1

The function of HSPs and their corresponding receptors is multiple. In the sense of innate immune response, HSPs directly stimulate the secretion of various inflammatory cytokines by APCs, the secretion of nitric oxide by macrophages and dendritic cells (DCs) and the secretion of chemotactic factor by macrophages, and stimulate the maturation of DCs and the migration of DCs to lymphoid tissue. HSPs, peptide chains from stimulated cells, together with major histocompatibility complex (MHC) molecules on the surface of APCs may result in re-presentation of the peptide chains on APC in an adaptive immune response [11-18]. Table 2 lists the immunomodulating properties of hyperthermia on immune reactions from different sources of reports. Basically, it is summarized as follows; a. fever-range temperature can modulate the activities of immune cells, including APCs, thymus cells (T cells) and natural killer cells (NK cells); b. heat shock temperature can increase the immunogenicity of tumor cells; c. cytotoxic temperature can create an antigen source for induction of an anti-tumor immune response.

The immunomodulatory effect of hyperthermia promotes an interest in hyperthermia aided immunotherapy, especially against tumors. There appears the notion of hyperthermia therapy as a type of medical treatment in which body tissue is exposed to slightly higher temperatures to damage and kill cancer cells or to make cancer cells more sensitive to the effects of radiotherapy and certain anti-cancer drugs. When combined with radiotherapy, hyperthermia is particularly effective at increasing the damage to acidic, poorly oxygenated parts of a tumor [19] and cells that are preparing to divide [20]. Hyperthermia treatment is
Table 2  Immunomodulating properties of hyperthermia [4]

<table>
<thead>
<tr>
<th>Hyperthermia level</th>
<th>Immunomodulating properties</th>
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<tbody>
<tr>
<td>Fever-range</td>
<td>Increase antigen uptake and phagocytosis by DCs and macrophages</td>
</tr>
<tr>
<td>temperature (39–40 °C) and heat</td>
<td>Stimulate antigen processing by increasing the expression of immunoproteasome, LMP2 and LMP7</td>
</tr>
<tr>
<td>shock temperature (41–43 °C)</td>
<td>Augment cross-presentation in DCs and prime CD8 T cells to produce CTLs</td>
</tr>
<tr>
<td></td>
<td>Induce activation and maturation of DCs</td>
</tr>
<tr>
<td></td>
<td>Enhance the migration of DCs to draining lymph nodes</td>
</tr>
<tr>
<td></td>
<td>Increase expression of TLR-4</td>
</tr>
<tr>
<td></td>
<td>Up-regulate the expression of MHC Class II , CD80, CD86 and CD40 in DCs</td>
</tr>
<tr>
<td></td>
<td>Promote the migration of lymphocyte to lymph and tumor tissue</td>
</tr>
<tr>
<td></td>
<td>Regulate the survival and persistence of lymphocyte in peripheral tissues by inducing the degradation of c-FLIP</td>
</tr>
<tr>
<td>Cytotoxic temperature (above 43 °C)</td>
<td>Enhance the function of effector T cells: increase the expression of CD95L in CTLs and augment the migration and lysis of NK cell</td>
</tr>
<tr>
<td></td>
<td>Increase the expression of MHC Class I on the surface of tumor cells</td>
</tr>
<tr>
<td></td>
<td>Up-regulate the expression of tumor antigens</td>
</tr>
<tr>
<td></td>
<td>Induce the expression of HSPs to provide “danger signal” for DC activation</td>
</tr>
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<td></td>
<td>Enhance the susceptibility of tumor cells to the lysis of CTL-mediated</td>
</tr>
<tr>
<td></td>
<td>Create an antigen source for induction of an anti-tumor immune response</td>
</tr>
</tbody>
</table>

Note: LMP—low molecular weight polypeptide; CTL—cytotoxic T lymphocytes; c-FLIP—cellular FLICE (Fas-associated death domain (FADD)-like interleukin-1-β-converting enzyme)-like inhibitory protein

the most effective when provided close enough with the radiotherapy. Hyperthermia treatments in conjunction with radiotherapy have been used in patients with early stage cancers of the breast, head and neck, and prostate. The complete response rates recorded by Bicher et al. were 82 % for breast cancer patients, 88 % for head and neck cancer patients, and 93 % for prostate cancer patients; projected 5-year survival rates were 80 %, 88 % and 87 %, respectively [21]. Some of the clinical trials based on HSP vaccine showed a longer disease-free survival time, a lower rate of recurrence and most importantly a strong immune response against tumor.

4  The application of hyperthermia in the treatment of human papilloma virus-infected skin diseases

Skin plays a role of protection. The protective roles are played in both passive and active ways. By means of passive protection, the physiochemical properties of skin resist the exogenous harmful influence. The active protection of skin is determined by its distinctive position in immunology. In 1986, Bos et al. proposed the term of skin immune system (SIS) as the definition of the complexity of immune response-associated cells presenting in normal human skin [22]. The SIS includes Langerhans cells (LCs), other antigen presenting cells (APCs), keratinocytes (KC), lymphocytes, endothelial cells, mast cells and many humoral factors.

The effect of hyperthermia on SIS is of particular interest due to that skin is readily accessed to for exogenous hyperthermia. It has been reported that transient local hyperthermia promoted the migration of epidermal LCs in a temperature-dependent manner, and increased the percentage of LCs with maturation markers in the migratory portion. These phenomena are more pronounced in human papilloma virus (HPV)-infected skin in an organic in vitro culture system [23]. Human skin treated by hyperthermia of temperatures over 41 °C exhibited increased signals of apoptosis, especially in HPV-infected skin [24], and the hyperthermia induced production of interferons (INFs) in HPV-infected samples [25]. In a recent observation, we noted that local hyperthermia at 44 °C could promote the infiltration of both CD4+ and CD8+ T cells in the lesion of condyloma acuminatum [26]. Hyperthermia therapy made LCs of mouse exhibited stronger potency to stimulate the proliferation of CD8+ T cells, while the effect was insignificant on CD4+ T cells (unpublished data). In a classical murine hypersensitivity reaction model, we noted that the action time of local hyperthermia applied to the sensitization sites affected the outcome at the elicitation stage, and in that local hyperthermia pre-applied to the sensitization site suppressed the subsequent intensity of hypersensitivity reaction, while concurrently applied or applied two days after sensitization increased the intensity of the reaction [27]. The above-mentioned and other studies suggest that hyperthermia could influence the activity of SIS, both at cellu-
lar and molecular levels.

Hyperthermia has been used in the treatment of deep fungal infection, bacterial infection and viral infection of skin. But most of the observation was based on case reports or case series. HPV is a ubiquitous family of viruses with more than 120 genotypes. Some HPV genotypes are potentially oncogenic (the so-called high risk types), and are the leading infectious cause of cervical cancers [28]. The discovery of oncogenic HPVs soon led to the production and application of virus vaccines which showed dramatic effect in prevention of this notorious condition in women, albeit its effect on treatment of established cancer or prevention of infection by other genotypes of HPV was in doubt. HPV-infected skin or mucosa is one of the most common symptoms in dermatology. Treatments of HPV infections include destructive therapy, antiviral therapy, antimitic therapy, immunotherapy, or combinations of these methods. Yet the efficacies of these therapies are variable. We have conducted a series of open or controlled trials to treat skin HPV infection by local hyperthermia [29–32]. Most of the studies used local hyperthermia at 44 °C for 30 min. The protocol was three consecutive treatments in three days with subsequent two treatments in two days two weeks later [29]. Curative effect was observed in three months. More than half of the treated patients were cured, much superior to the control arm in the controlled trial. The method was easy to perform. Most of the patients tolerated the treatment well. A series of challenging clinical cases, such as giant lesions in patients with complication of diabetes mellitus, pregnancy, lesions with cosmetic sequelae or with superimposed skin conditions, were also successfully managed by this safe method [30–32]. In patients with multiple lesions, successful clearance of the target lesion is usually accompanied by the clearance of other untreated, remote lesions. This is one of the common findings in the above-mentioned clinical trials. This phenomenon, as well as the observation of effect of hyperthermia on immune cells, strongly suggests that local hyperthermia took effect by helping the establishment of specific immune response against HPV-infected cells.

5 Conclusion

In summary, hyperthermia affects a number of cellular and molecular constitutents in the SIS, involving both innate and adaptive immune responses. The impacts of hyperthermia on immune cells depend on the dosage (as well as the level) of hyperthermia, cellular types, cellular environment and even the developmental stages of cells. The efficacy of hyperthermia in treating some infectious and cancerous conditions has been validated and applied in clinics, while understanding the molecular mechanisms of hyperthermia on the immunereaction remains challenging. In clinical studies, the future directions for hyperthermia applications may rely on; a. advances in devices to deliver or measure uniform level and precise amount of heat; b. hyperthermia combined with gene therapy; c. combining with biologics; d. in combination with signal transduction pathway agonists or antagonists; e. in combination with other physical or chemical factors; f. other auxiliary factors. To elucidate the molecular mechanisms of hyperthermia on target cells, bio-omics studies, such as genomics, proteomics and transcriptomics, should be applied, in addition to employing the genetically modified cellular and animal models.

References


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