Seminars in Cancer Biology 37-38 (2016) 96–105

Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer

Review

Effects of hyperthermia as a mitigation strategy in DNA damage-based cancer therapies

Theodora Mantso[a], George Goussetis[b], Rodrigo Franco[c], Sotiris Botaitis[d], Aglaia Pappa[e], Mihalis Panayiotidis[a,*]

[a] School of Life Sciences, Heriot Watt University, Edinburgh, Scotland, UK
[b] School of Engineering & Physical Sciences, Heriot Watt University, Edinburgh, Scotland, UK
[c] School of Veterinary Medicine & Biomedical Sciences, University of Nebraska—Lincoln, USA
[d] Second Department of Surgery, Democritus University of Thrace, Alexandroupolis, Greece
[e] Department of Molecular Biology & Genetics, Democritus University of Thrace, Alexandroupolis, Greece

ARTICLE INFO

Article history:
Received 10 February 2016
Received in revised form 25 March 2016
Accepted 25 March 2016
Available online 26 March 2016

Keywords:
Hyperthermia
DNA damage
DNA repair
Cancer therapeutics
Cancer treatment

ABSTRACT

Utilization of thermal therapy (hyperthermia) is defined as the application of exogenous heat induction and represents a concept that is far from new as it goes back to ancient times when heat was used for treating various diseases, including malignancies. Such therapeutic strategy has gained even more popularity (over the last few decades) since various studies have shed light into understanding hyperthermia's underlying molecular mechanism(s) of action. In general, hyperthermia is applied as complementary (adjuvant) means in therapeutic protocols combining chemotherapy and/or irradiation both of which can induce irreversible cellular DNA damage. Furthermore, according to a number of *in vitro*, *in vivo* and clinical studies, hyperthermia has been shown to enhance the beneficial effects of DNA targeting therapeutic strategies by interfering with DNA repair response cascades. Therefore, the continuously growing evidence supporting hyperthermia's beneficial role in cancer treatment can also encourage its application as a DNA repair mitigation strategy. In this review article, we aim to provide detailed information on how hyperthermia acts on DNA damage and repair pathways and thus potentially contributing to various adjuvant therapeutic protocols relevant to more efficient cancer treatment strategies.

Contents

* Corresponding author at: School of Life Sciences, Heriot Watt University, John Muir Building, Riccarton Campus, EH14 4AS, Edinburgh, Scotland, UK.
E-mail address: M.Panagiotidis@hw.ac.uk (M. Panayiotidis).

http://dx.doi.org/10.1016/j.semcancer.2016.03.004
1044-579X/

1. Introduction

Hyperthermia is defined as the application of exogenous heat induction and has been well known for its therapeutic effects against several diseases including malignancies. Since the 1970s and 80s, several studies have focused on understanding the biology behind hyperthermia's beneficial effects on various cancer treatments. Heat induction is most commonly applied in combination with other primary therapies (*e.g.* radiation therapy and/or chemotherapy) since it can enhance their therapeutic effectiveness [1–3]. Results from *in vitro* and *in vivo* studies have shown that hyperthermia in the range of 41–47 °C exerts various effects including i) direct killing of tumor cells, ii) alterations in the tumor microenvironment, iii) induction of heat shock proteins, iv) activation of the immune response, v) induction of the apoptotic cascade, vi) improvement of therapeutic outcome when applied with other treatments, vii) changes in cell cycle regulatory signaling pathways and viii) alterations in blood flow, oxygen and nutrients' distribution in the tumor site. From hot water pads to more technologically advanced hyperthermia platforms (utilizing different energy sources such as radiofrequencies, microwaves and laser), the main aim is to maintain the temperature elevation at the level of whole body (whole-body hyperthermia) and/or at the specific tumor site (local or regional hyperthermia) [4,5].

Earlier studies have demonstrated that apart from its cytotoxic properties, heat induction can also act as a "sensitizer" to other DNA damage-based treatment modalities. In fact, numerous studies have shown that hyperthermia in combination with radiation and/or various other chemotherapeutic drug treatments can induce significant antitumor effects under *in vitro* and *in vivo* experimental settings. Moreover, such studies have suggested that heat induction can cause DNA damage directly [6] and/or interfere with DNA repair pathways [7] thus enhancing the outcome of antineoplastic strategies. To these ends, hyperthermia not only affects a single DNA repair pathway but rather many different ones thus resulting in the accumulation of damage lesions which in turn leads to cell death. Therefore, although enhancing our understanding of the underlying mechanisms by which hyperthermia can influence DNA damage is quite intriguing, it is also of great importance when looking into improving current and designing new, novel and innovative therapeutic approaches. Finally, this review article aims to describe the effects of hyperthermia as a mitigation strategy in DNA damage-based cancer therapies due to its ability to influence DNA damage and repair pathways and how such knowledge is employed in order to establish adjuvant cancer treatments of greater therapeutic effectiveness.

2. Hyperthermia and DNA damage response

2.1. Direct effects

The prospect of hyperthermia inducing DNA damage directly rather than by other indirect means (*i.e.* ROS generation) has been the subject of several studies. Results from initial reports demonstrated that heat elevation can lead to the generation of DNA breaks after exposing Chinese hamster ovary cells to 45 °C [6]. Additional studies using HeLa cells have showed that heat treatment at 43 °C resulted in the induction of DNA lesions observed as early as a 15 min of exposure. Moreover, it was evident that there was a strong correlation between the amount of hyperthermia-induced DNA strand breaks and cytotoxicity [8]. In an attempt to further characterize DNA breakage (in CHO cells following heat treatment), elevated levels of apurinic/apyrimidinic or abasic sites were observed [9]. In addition, work by other groups has suggested that although hyperthermia cannot induce the generation of double strand breaks (DSBs) by itself, it can alter the rates of observed DSBs (in radio-sensitization experiments) by increasing the levels of slowly rejoined DSBs instead [10]. Furthermore, experiments with primary human fibroblasts have also demonstrated that hyperthermia does affect the kinetics of DNA repair mechanisms by considerably slowing down cells. Elevated levels of mis-rejoined and non-repairable DSBs were accompanied by a drop in the rates of successfully completed DNA repair after heat exposure in combination with irradiation. To this end, it was supported that hyperthermia by promoting delayed DNA repair processes can have an impact on the observed increase on levels of DNA breaks, after irradiation, which if not correctly repaired they will either stay non-rejoined or rejoin with a wrong broken end. It has been postulated that the latter hypothesis is more likely to occur due to their higher detection levels [11].

Evidence from several hyperthermia-induced radiosensitization studies has shown that heat induction can affect DNA repair pathways as it restricts replication by reducing the activity of DNA polymerases, thus eventually increasing the appearance of more DNA breaks after irradiation [12–14]. In addition, data from early research reports suggested that heat treatment can promote topoisomerase inactivation in HeLa S3 cells [15] while other studies conducted in human epidermoid cancer (KB) cells have reported increased transcription and translation levels of topoisomerase II after exposure to 42 °C or 45 °C. To this end, treatment of KB cells with the etoposide VP-16 (a topoisomerase II targeting drug) was accompanied by further potentiation of cytotoxicity following these hyperthermia exposures [16]. The nature of these contradicting results (*e.g.* decreased enzyme activity or upregulation of expression levels of topoisomerase II) may be the end result of variations in experimental conditions regarding the duration and/or temperature of exposures in addition to the usage of different cell types. On the other hand, later research reports have outlined the induction of DSBs along with focal phosphorylation of histone H2AX (at Ser139) in response to heat treatments in H1229 cells. In general, γH2AX foci are considered to be indicators of DSBs [17] whereas other studies have addressed the importance of H2AX's role in recruiting several repair factor molecules to areas exposed to DNA damage [18]. A number of studies investigating the induction of γH2AX foci formation by hyperthermia have resulted in the publication of opposing results. For instance, some of these studies support a direct association of γH2AX foci with DSBs whereas other findings support an indirect role where H2AX phosphorylation occurs due to other cellular processes being "disturbed" by hyperthermia. The generation of γH2AX foci was found to increase linearly in an exposure duration-dependent manner when exposing cells at temperatures from 41.5 °C to 45.5 °C. In addition, the contribution of each cell cycle phase to enhancing the formation of γH2AX foci was studied with cells in the S phase exhibiting significantly more potential compared to those at the G1 or G2-M phase, thus suggesting the involvement of DSBs induction in triggering hyperthermia-induced cell death pathways [19]. Moreover, findings from subsequent studies confirmed that hyperthermia can promote the formation of γH2AX foci, whilst also revealing the dependence of this process on