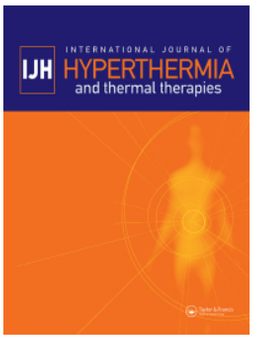

International Journal of Hyperthermia

ISSN: 0265-6736 (Print) 1464-5157 (Online) Journal homepage: informahealthcare.com/journals/ihyt20

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Ronald A. Coss

To cite this article: Ronald A. Coss (2005) Inhibiting induction of heat shock proteins as a strategy to enhance cancer therapy, International Journal of Hyperthermia, 21:8, 695-701, DOI: 10.1080/02656730500331918

To link to this article: https://doi.org/10.1080/02656730500331918

Published online: 13 May 2011.

Int. J. Hyperthermia, December 2005; 21(8): 695–701

Taylor & Francis
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Inhibiting induction of heat shock proteins as a strategy to enhance cancer therapy

RONALD A. COSS

Department of Radiation Oncology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA, USA

(Received 23 May 2005; accepted 31 August 2005)

Abstract
Cancer treatments that incorporate thermal therapy and some systemic therapies induce the production of heat shock or stress proteins. The induced heat shock proteins could lessen the effect of the therapy by inhibiting apoptotic signaling and by acting as molecular chaperones to prevent irreversible cellular damage. Strategies that prevent the induction of heat shock proteins would result in more apoptosis and necrosis, improving the cancer therapy. This paper briefly reviews cancer therapies that induce the stress response, and proposes strategies to reduce the stress response.

Keywords: *Heat shock proteins, apoptosis, acute acidification, quercetin, cancer therapy*

Hypothesis

Thermal therapy induces damage to protein and membrane structures in cells leading to cell death [1–3] (Figure 1). Cells undergo apoptotic and/or non-apoptotic death [4] following thermal therapy and the mode of death is dose and cell type-specific. Thermal therapy also induces the stress response, characterized by induction of heat shock proteins (HSPs). HSPs are molecular chaperones that prevent irreversible inactivation of proteins and target denatured proteins for proteolysis [5, 6]. HSPs also are a class of inhibitor of apoptosis proteins (IAPs) that block apoptotic signalling, inhibit apoptosis and enhance survival [6–9]. Strategies that inhibit the stress response induced by thermal therapy would, therefore, enhance cytotoxicity resulting from both modes of cell death. Inhibition of the stress response would enable apoptosis and/or enhance necrosis. The strategy of inhibiting the stress response is applicable to any modality (e.g. thermal therapy, chemotherapy, radiation therapy) that kills tumour cells through induction of apoptosis as well as necrosis.

Correspondence: Ronald A. Coss, PhD, Department of Radiation Oncology, Thomas Jefferson University, 111 S. 11th Street, Philadelphia, PA 19107-5097, USA. Tel: (215) 955-1056. Fax: (215) 955-5825. E-mail: ronald.coss@mail.tju.edu

ISSN 0265–6736 print/ISSN 1464–5157 online
DOI: 10.1080/02656730500331918

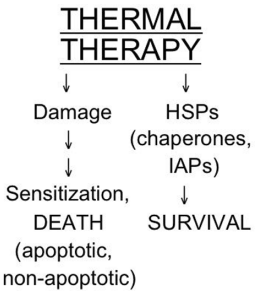

Figure 1. This diagram illustrates that thermal therapy induces damage to various cellular structures and induces the production of heat shock proteins (HSPs). The cellular damage can sensitize the cell to other modalities and result in apoptotic and/or non-apoptotic death, depending on the dose and cell type. HSPs have a dual function. They interact with affected proteins to prevent their irreparable damage and allow for cell survival. HSPs also act as inhibitor of apoptosis proteins (IAPs) to block apoptotic signalling and inhibit proteolysis of critical targets, leading to cell survival. Therefore, preventing the induction of HSPs will (i) prevent repair of damaged cell structures and (ii) enable apoptotic signalling and apoptosis. The end result is enhanced cell death.

Inhibition of the stress response by acute intracellular acidification

Human melanoma cells cultured at acidic pH, a characteristic of regions of solid tumours that may affect treatment outcome [10–14], are resistant to thermal therapy compared to cells cultured at pH 7.3 [15–17]. This is due in part to elevated endogenous levels of HSPs [15, 17]. Acute extracellular acidification inhibits the 42°C-induced stress response and sensitizes the melanoma cells to 42°C [15, 17]. The accompanying reduction of intracellular pH rather than extracellular pH appears to be the critical factor for hyperthermia sensitization [16, 17].

Intracellular pH thresholds were found to exist for melanoma cells growing at pH 7.3 below which the stress response was inhibited and cells were sensitized to 42°C. In contrast, intracellular pH thresholds for heat sensitization did not exist for cells growing at pH 6.7: any reduction in intracellular pH prior to heating resulted in increased cell killing [17]. Since cells grown at low pH lack an intracellular pH threshold for heat sensitization, they are sensitized more to 42°C per unit decrease in intracellular pH than cells grown at pH 7.3, the extracellular pH characteristic of most normal tissues. A reduction of intracellular pH to 6.5 or lower was required to sensitize DB-1 melanoma cells cultured at pH 6.7 to thermal therapy [16]. This is easy to accomplish *in vitro* by changing the pH of the growth medium. It is not as easy to acidify tumours.

The extracellular pH of human tumours subjected to hyperglycemia can be transiently reduced by an average of 0.2 pH unit, while the extracellular pH of normal tissues remain unchanged [18–22]. However, this degree of acidification is not enough to sensitize human tumour cells to hyperthermia [15–17]. Additional strategies need to be employed to enhance tumour acidification for sensitization to hyperthermia. Melanoma cells rely on monocarboxylate transporters (H+ lactate symporters) to remove hydrogen ions under acidic extracellular conditions [23]. A combination of mild acute acidification with an inhibitor of MCTs reduced intracellular pH enough to selectively sensitize melanoma cells cultured at pH 6.7 to hyperthermia by lowering their intracellular pH below the critical threshold value by a treatment that does not lower the intracellular pH below the critical threshold of cells growing at pH 7.3 [16]. It has also been shown that extracellular pH of tumours can be decreased below pH 6.3 by inhibition of MCTs and/or inhibition

of mitochondrial respiration by site 1 respiration inhibitors during hyperglycemia [24, 25]. These findings support the concept for sensitization of human melanoma cells existing in a chronic acidic environment to hyperthermia by strategies that selectively and acutely lower the intracellular pH in acidotic regions of tumours.

HSPs inhibit apoptosis

The proposed strategy of inhibition of the stress response is applicable not only to thermal therapy but also to other modalities used for treating cancer, especially those that induce apoptosis. The heat shock proteins HSP70, HSP27 and HSP90 have been shown to inhibit apoptotic signalling and reduce apoptosis in different model systems [26–29]. Numerous excellent reviews, including those cited previously [6–9], discuss the importance of HSPs as modulators of apoptosis. Therefore, treatment strategies that reduce HSP levels and prevent the induction of HSPs by a therapy should enhance tumour response.

Phase I and II trials are underway with Hsp90 inhibitors, especially the geldanamycin analogue 17-(allylamino)-17-demethoxygeldanamycin (17-AAG) [30–33]. Disruption of Hsp90 function leads to dissociation and loss of function of many of this chaperone's 'oncogenic client proteins' crucial for tumour cell survival [34]. However, this class of benzoquinone ansamycin antibiotics also induces the stress response [35, 36]. The same is true of other Hsp90 inhibitors including the macrocyclic anti fungal antibiotic radicicol [37]. The farnasyltransferase inhibitor manumycin being considered for treatment of mesothelioma and ovarian cancer [38, 39] also induces the stress response [39, 40]. Therefore, pre-treatment with another agent that reduces the stress response should further enhance apoptosis and the effectiveness of these systemic therapies.

Systemic strategies to reduce the stress response

Systemic strategies that could be used to reduce the endogenous expression of HSPs and inhibit therapy-induced stress responses include anti sense strategies that target HSPs and drug intervention strategies using the flavonoid quercetin or the inhibitor KNK437. The use of quercetin is especially promising.

Quercetin is a broad spectrum inhibitor that interferes with the binding of HSF-1 to heat shock promoters, thereby inhibiting the stress-induced synthesis of HSP mRNAs [41, 42]. Quercetin also is an anti oxidant and a modulator of signalling pathways [43] and is more effective under acidified conditions [44–46]. Quercetin has been shown to significantly sensitize human prostate xenografts to thermal therapy-induced tumour growth delay [47]. Furthermore, the continuous treatment with quercetin over a 5-week period was not toxic to the host nude mice [47]. Separate studies have demonstrated that exposure of prostate tumour cell lines to quercetin or to anti-sense oligonucleotides *in vitro* led to depletion of HSP70 expression and apoptosis in the absence of thermal therapy [48]. Furthermore, pretreatment of the prostate cell lines with quercetin synergistically enhanced apoptosis induced by thermal therapy [48]. Pretreatment with quercetin has also been shown to reduce the induction of HSP70 by the farnesyl transferase inhibitor manumycin in ovarian cancer cell lines and mesothelioma biopsies and to enhance apoptosis [39]. The use of quercetin is translatable to the clinic: quercetin is used to treat chronic prostatitis [49, 50]. Furthermore, there is an increasing literature on the systemic effects of quercetin from the field of clinical nutrition [51, 52].

Less is known about KNK437. It is a compound developed by the Kaneka Corporation (Takasago, Japan). KNK437 inhibits the induction of HSPs *in vitro* and *in vivo* [53, 54].

Pre treatment with KNK437 enhanced thermally induced apoptosis in human cancer cell lines [55, 56].

A different approach to inhibit the stress response involves use of RNA interference technology to knock down specific HSPs. RNA interference is a rapidly emerging and powerful technique used to investigate gene function by degrading a specific mRNA target in a cell or organism and, thus, knocking out or knocking down the level of the encoded protein. The specific mRNA degradation is mediated by complementary double-stranded RNA [57–59]. The use of RNA interference molecules to target HSP70, HSP27 and HSP90 mRNAs may abrogate the therapy-induced stress response. The utility of RNA interference technology for the treatment of cancer remains to be determined.

Impact on the immune response

HSPs are known to enhance and play a role in the immune response elicited against tumour cells (see separate articles in this issue on HSPs and the immune response by Calderwood and Subjeck). Therefore, strategies that decrease treatment induced expression of HSPs may also reduce the immune response directed against the tumours. This cannot be tested using human xenografts, since the rodent hosts (SCID or nude mice) do not have competent immune systems. Strategies that partially but not completely inhibit the treatment induced stress response may allow for increased apoptosis and for an enhanced immune response. Alternatively, strategies that reduce expression of Hsp27 or Hsp70 but not Hsp90 or Hsp110 may also accomplish the same result. Hsp90 [60] as well as Hsp70 [61] have been shown to be expressed on the surface of melanoma cells and are potential immunorelevant targets for immunotherapy [60]. Strategies that target the surface expressed Hsp90 with cell impermeable analogues of geldanamycin also are being considered [33].

Future studies

Future studies require the testing and confirmation of the proposed strategy in multiple tumour models *in vitro* and *in vivo*. Potential agents considered for reduction of the stress response must be capable of being used systemically and/or locally. The potential agents must be capable of reducing the levels of HSPs and inhibiting the induction of HSPs, without inducing normal tissue damage. Studies using rodent models with competent immune systems must also be carried out to determine the effect of inhibiting the stress response on tumour growth delay. A therapeutic gain must be demonstrated *in vivo* before Phase I trials are to be considered.

Acknowledgements

This work was supported by NIH grants PO1 CA56690 and P30 CA56036. Thanks are expressed to the Society for Thermal Medicine for support of this Workshop.

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