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Effect of whole-body hyperthermia on hepatic cytochrome P450

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Summary. Currently, the combination of hyperthermia with a variety of chemotherapeutic agents and cytokines for the treatment of disseminated human malignancy is being examined. In this study we investigated the effects of 41° – 42° C whole-body hyperthermia (WBH) and the cytokine interleukin-1 (IL-1) on cytochrome P450 in mice. At 24 h following 1 h of 41° – 42° C WBH, IL-1 or combined treatment, hepatic microsomal cytochrome b_5 and aminopyrine *N*-demethylation were assayed. Cytochrome b_5 activity was not significantly diminished by WBH, IL-1 or WBH + IL-1, but *N*-demethylation was suppressed by the combination of WBH + IL-1 and, to a lesser extent, by WBH alone.

Introduction

Hyperthermia is currently being examined as a treatment modality for cancer since it appears to act synergistically with other modalities such as radiation and chemotherapy and yet does not share the same spectrum of toxicities [9]. The systemic nature of most cancers refractory to conventional therapy underlines the importance of investigating the development of systemic (i. e. whole-body) hyperthermia (WBH) as a possible adjunctive modality of cancer treatment.

Many chemotherapeutic agents appear to demonstrate additive or supra-additive antineoplastic activity when combined with hyperthermia in vitro and in vivo [8, 9, 12]. Extrapolation to the clinical WBH setting would be hazardous if hyperthermia directly or indirectly interfered with drug metabolism. Interleukin 1 (IL-1) or endogenous pyrogen [6], which is derived from a variety of cells including macrophages and epidermal cells, shows increased activity at elevated temperatures [3]. In addition, we have recently demonstrated increased IL-1 production during WBH in mice, with peak levels of IL-1 mRNA occurring 16–24 h following a 1-h, 41° – 42° C WBH treatment [13]. We hypothesized that WBH might suppress hepatic microsomal enzyme activity, with IL-1 acting as an intermediary. We therefore studied the effect of a single, 1-h, 41° – 42° C WBH treatment, IL-1 treatment alone or combined WBH-IL-1 treatment on hepatic drug metabolism. This is the first study that has examined the effect of WBH on drug metabolism.

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Materials and methods

WBH. We recently developed a simple technique for accomplishing 41° – 42° C WBH in unrestrained unanesthetized mice using a radiant heat device (Enthermics Medical System; Menomonee Falls, Wis) [18]. An elevated wall temperature was accomplished in this study by an electrical thermal cable system that produced a low-power-density, radiant heat. A plastic animal cage was inserted into the stainless steel device, the spacing of the heating elements producing a stable air temperature throughout the holding cavity with minimal convective heat losses.

Prior to WBH, all animals received 0.5 ml sterile normal saline intraperitoneally to prevent dehydration. Once color-coded, the mice were placed in the plastic cage insert of the radiant heat device before it was switched on. Rectal temperatures were taken by inserting a thermocouple probe every 10 min. Time zero was defined as the time when the average rectal temperature of the mice in the device was 41° C (range, 40.5° – 41.5° C), and the 1-h duration of WBH was recorded from this time. Treatments were terminated by removing the animals from the device and allowing them to cool down at room temperature.

Manipulation of the ambient air temperatures (42° – 45° C) by opening and closing the heating-device drawer enabled us to heat the mice reliably and safely to 41° – 42° C. However, we found that one or more of the animals occasionally "overshot" the 42° C maximal rectal temperature. In this instance, the animal was removed from the device and allowed to cool to 40° C before being returned to the heating device.

WBH and IL-1 treatment. A total of 24 C57B1/6J mice (Jackson Laboratories) were divided into four groups: A, control; B, IL-1; C, WBH and D, WBH + IL-1. Prior to WBH, the mice received 0.5 ml PBS or 0.5 ml (200 ng/ml) IL-1 in PBS. The IL-1 was kindly supplied by Dr. D. Sauder, McMaster University. Groups C and D then underwent 41° – 42° C WBH for 1 h, whereas groups A and B underwent a sham WBH treatment, i. e. they were placed in a similar container and had their rectal temperatures measured every 10 min.

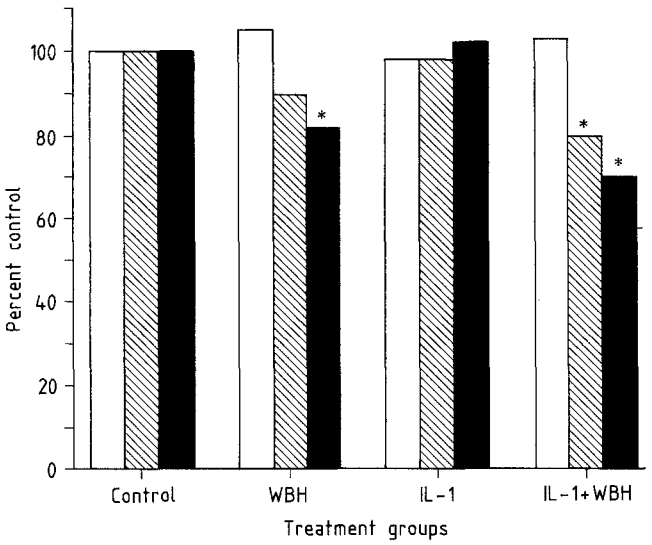

Fig. 1. Cytochrome b_5 and P450 levels and *N*-demethylation enzyme activity in mice. For clarity, the results are expressed as a percentage of control values and error bars are not shown. Control cytochrome b_5 = 0.067±0.017 nmol/mg protein (mean ± 2 SD); control cytochrome P450 = 1.187±0.38 nmol/mg protein; control *N*-demethylation = 430±90 nmol HCHO/h per mg protein. *, $P < 0.05$

Enzyme assays. Mice were sacrificed 24 h post-WBH. Their livers were removed and microsomes were prepared by differential centrifugation. Microsomal cytochrome P450 and b_5 levels were determined by difference spectroscopy [14]. Aminopyrine *N*-demethylation was determined by the measurement of formaldehyde (HCHO) formed [21]. Protein determination was done by the Lowry assay [11].

Statistical analysis. Replicate levels of cytochrome P450 (expressed as nmol/mg protein) and its activity measured by *N*-demethylation of aminopyrine (expressed as nmol HCHO formed/h per mg protein) were assumed to conform to a normal distribution; therefore, two-sample *t*-tests (two-tailed) were carried out to compare the means of each group ($n = 6$ per group).

Results

There was no acute IL-1- or WBH-related toxicity in any of the mice. Cytochrome b_5 and P450 levels and *N*-demethylation enzyme activity for each group are shown in Fig. 1. There was clearly no significant effect of single or combined modality treatment on cytochrome b_5 levels. However, *N*-demethylation of aminopyrine was significantly reduced by WBH + IL-1 treatment and, to a lesser extent, by WBH alone. At the dose used in this study, IL-1 alone did not reduce *N*-demethylation activity.

Discussion

Recent interest in the use of hyperthermia in the treatment of disseminated human malignancy has focussed on its adjunctive role rather than on its use as a single modality therapy [19, 20]. Hyperthermia has shown evidence of synergistic antineoplastic effects with a variety of antineoplastic agents [6, 8, 12], labilizing agents [1, 17, 22] and biological products, including interferons [2]. There is now evidence that biological response modifiers such as the interferons and the cytokine IL-1 are not only associated with endogenously elevated or febrile temperatures but also show increased activity when exogenous hyperthermia is applied [3].

Other investigators [7] have previously shown depression of drug-metabolizing enzymes and microsomal P450 by bacterial endotoxins. Such endotoxins have many properties now recognized as belonging to the macrophage-derived cytokine IL-1 [4]. Ghezzi et al. [6] could demonstrate depression of liver drug metabolism by recombinant IL-1, although the doses used that produced statistically significant differences were 2,500–5,000 ng, considerably in excess of the 100-ng doses used in the present study. We did not show a significant inhibition of *N*-demethylation of aminopyrine by IL-1 alone in our in vivo experiments. The dose of 100 ng, however, was chosen on the basis of previous work conducted in our laboratory and by other workers [10], who have demonstrated significant biological activity at these dose levels.

When combined with the same 100-ng dose of intraperitoneal IL-1, a single, 1-h, 41°–42° C WBH treatment produced significant depression of *N*-demethylation activity. WBH alone reduced enzyme activity to a lesser extent. We have previously demonstrated that a similar, single WBH treatment in mice can induce mRNA for cutaneous IL-1 [13]. It seems reasonable to speculate that other tissues would respond in a similar fashion. We did not measure IL-1 levels in the present study of WBH-treated mice, and we cannot exclude the potential heat-induced participation of other biological products such as interferons, which have clearly been shown to depress hepatic drug metabolism [15]. In addition, Ghezzi et al. [5] have also published evidence from in vivo studies showing that tumour necrosis factor (TNF) reduced several hepatic drug-metabolizing enzymes. Since TNF was not active on isolated rat hepatocytes in vitro, it was suggested that a second mediator such as IL-1 might be involved [5]. Whether or not the effect of 41°–42° C WBH is mediated through IL-1, the inhibitory effect of IL-1 on *N*-demethylation was significantly augmented by hyperthermia in our studies. It appears reasonable to conclude from our results that the effects of WBH and IL-1 were not simply independent, given the negligible effect of IL-1 alone.

The mediators of hyperthermia- or IL-1-induced perturbations of hepatic drug metabolism are unknown. Our studies do not distinguish between increased degradation and decreased synthesis of enzymes; indeed, both situations may apply. Some investigators [6] have even suggested that interferons, for example, increase the degradation of cytochromes, perhaps through the generation of reactive oxygen intermediates to which microsomal cytochrome P450 is exquisitely sensitive [6]. Hyperthermic treatment of cells is associated with the development of a variety of "heat-shock" proteins, the function of which is only partly understood. It seems reasonable to hypothesize that certain heat-shock proteins may play a regulatory role in the transcription of drug-metabolizing enzymes. IL-1 activity is associated with liver cell production of acute-phase proteins and decreased expression of proteins such as albumin [16]. A common link may therefore exist between hyperthermia and IL-1-induced proteins in view of the hyperthermic potentiation of Il-1 activity demonstrated in the present study.

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