Eur J Cancer Clin Oncol, Vol. 20, No. 8, pp. 1079–1085, 1984.
Printed in Great Britain.

0277-5379/84$3.00+0.00

Production of Systemic Hyperthermia in the Rat*

PETER F. LORD,†‡ DANIEL S. KAPP,§ TIMOTHY HAYES‡ and ZEEV WESHLER§

†*Department of Diagnostic Imaging,* ‡*Section of Comparative Medicine and* §*Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, CT* 06510, *U.S.A.*

Abstract—*The design of clinical trials employing whole-body hyperthermia in cancer therapy has been hampered due to lack of a suitable animal model. We describe a technique for reproducibly and efficiently inducing whole-body hyperthermia in Sprague–Dawley rats, using halothane and oxygen anesthesia and immersion in a hot water bath. Core body temperatures of between* 41.5 *and* 43°C *were induced and maintained for periods of up to* 200 min *and survival curves were determined. The time of exposure at a given temperature that resulted in death in* 50% *of the animals within* 24 hr *after heating* ($LD_{50/24\,hr}$) *was calculated by linear logistic regression analysis.* $LD_{50\ 24\ hr}$ *values of* 115, 61, 57, 25 *and* 16 min *were obtained for temperatures of* 41.75, 42.0, 42.25, 42.5 and 42.75°C *respectively. This heating technique is compared to several more toxic methods for inducing whole-body hyperthermia with respect to possible pharmacological and physiological differences.*

INTRODUCTION

WHOLE-BODY hyperthermia is being used either alone or in combination with chemotherapy and radiotherapy in phase I and phase II trials in the treatment of deep-seated and disseminated neoplasia [1–6]. Temperatures of 41.8°C or more for several hours are usually employed and treatments are fractionated. Whole-body hyperthermia has not only the potential for local tumor control but, because of its systemic effect, may be effective alone or with drugs against metastases. In contrast to local hyperthermia, there has been little research in the field of systemic hyperthermia employing laboratory rodents because investigators have not been able to keep rodents alive following exposures to clinically relevant times and temperatures. Studies have been done on larger animals, including the dog [7–9] and the pig [10]. Rodents have been considered to tolerate heating poorly [11]. Dickson and Muckle [12] heated anesthetized rabbits by radiant heating in an insulated box but could not maintain a constant intra-abdominal temperature—an average temperature of only 40.8°C was achieved. Even local heating of large tumors on the legs of rats raised the body temperature to fatal levels [13–15]. Yerushalmi [16] heated mice but could not maintain a temperature of 42°C for a significant time. The mortality of Buffalo rats treated with systemic hyperthermia by immersion was 100% at 42°C for 15 min [17]. None of the core body temperatures obtained were within the 41.5–43°C range considered to have a tumoricidal effect [18, 19]. A reliable method of heating rodents to these temperatures is needed to study fractionated whole-body hyperthermia in conjunction with chemotherapy and/or radiation therapy [20, 21].

We report here a method of producing systemic hyperthermia in the anesthetized rat using immersion in a temperature-controlled water bath.

MATERIALS AND METHODS

Female Sprague–Dawley rats of 200–225 g weight were sedated with 8–10 mg ketamine hydrochloride (approximately 50 mg/kg) i.m. and anesthetized with 1% halothane and oxygen. A tightly fitting face mask comprised of a small plastic beaker with a rubber seal made from a surgical glove was placed over the animal's head. The gas was delivered to the mask by plastic tubing from a standard anesthesia machine. Inspiratory and expiratory valves were made from test tubes containing water to a level of about 3 cm

Accepted 14 October 1983.
*This work was supported by Grant CA06519 from the National Cancer Institute, Grant IN-31-0-7 from the American Cancer Society, Grant PHS PO 6 RR00393 from the American Resources Branch, NIH, and VS PHS Grant CA16359-06.

and were used to control the flow of gas to each rat [22]. A glass tube was inserted below the water level to form a seal. A distribution jar was connected to the anesthesia machine which allowed up to four rats at a time to be anesthetized (Fig. 1) [22]. The gas flow to four rats was 1 l/min, just enough to supply each animal with fresh gas on demand. The minimum concentration of halothane which could keep the rats anesthetized was used (usually 1%). Drawing on our early experience with dogs [8], we found that when core temperatures of 42°C or more had been reached little or no halothane was needed.

The anesthetized rats were placed in paper slings made of surgical face masks. The ties of the masks were taped onto the sides of a controlled-temperature circulating waterbath (Temptrol Model No. 255, Precision Scientific Company) so that the rats were partly immersed in the water (Fig. 1). The degree of immersion was controlled by tightening or loosening the ties to raise and lower the animals. This enabled the rats' body temperature to be regulated by the degree of immersion of the tail and hind quarters. It was necessary to monitor the temperatures continually and adjust the slings when the temperature deviated more than 0.1°C from the desired temperature.

The core temperature of each rat was monitored by a thermoprobe (Yellow Springs Instruments Co.) inserted to a distance of at least 6 cm up the rectum and taped to the tail. The probes were calibrated against a mercury thermometer traceable to the National Bureau of Standards and accurate to 0.1°C. The waterbath temperature was set at the same temperature at which the rats were to be maintained. To produce a rapid rise in temperature, the rats were almost totally immersed except for their heads and feet until they reached the desired temperature. After the treatment, the rats were removed from the waterbath, thoroughly dried with a towel and placed in a closed plastic box on a water-heated pad at 39–40°C. This was done in order to prevent the sudden hypothermia that the rats developed if they were not protected from cold until they regained consciousness.

Groups of ten or more rats were treated to temperatures of 41.5°C from 90 to 180 min, 41.75°C from 60 to 180 min, 42.0°C from 30 to 90 min, 42.25°C from 30 to 90 min, 42.5°C from 10 to 60 min and 42.75 °C from 5 to 30 min. The duration of exposure was measured from the moment each rat reached the desired temperature. Core temperatures were recorded every 5 min after all the rats in the waterbath had been anesthetized

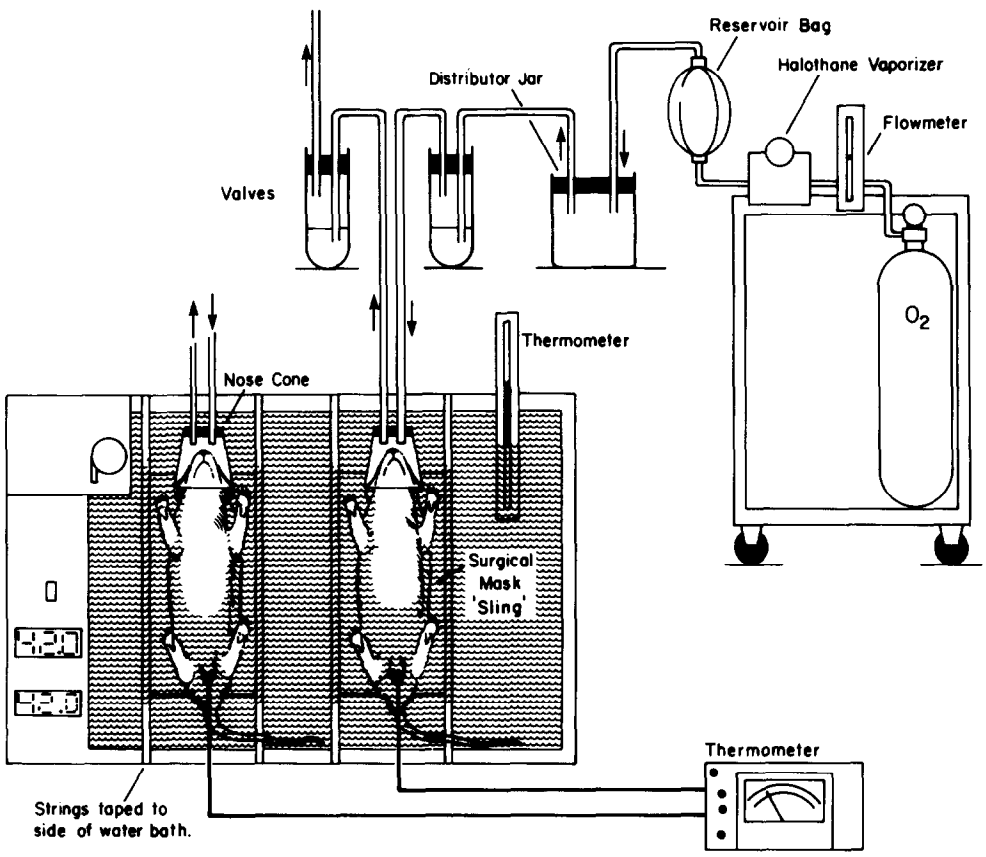

Fig. 1. Diagram illustrating method of anesthetizing and heating up to four rats at a time with continuous monitoring of their core (rectal) temperature. Only one set of valves and tubes supplying the anesthetic gases is shown for simplicity.

and positioned in the slings. The survival was measured at 24 hr after heating. Temperatures of two additional groups of rats heated at 41.75 and 42.5 °C were monitored for 3 hr from completion of sublethal heating until the rats recovered consciousness. As a further control, a group of 12 rats was maintained at 37.1°C for 1 hr in the waterbath and the temperatures recorded during recovery from this sham heating.

Other investigators have given rats an i.p. injection of Ringer's lactate solution to replace water lost during treatment [17]. We compared survival of groups of rats treated with and without an i.p. injection of 20 ml/kg Ringer's lactate solution.

Additional groups of rats were anesthetized with 25 mg/kg pentobarbital sodium, which produced light general anesthesia. One group of 12 rats was heated to 41.75°C for 1 hr and another group of 12 to 42.5°C for 25 min.

A linear logistic regression model was used to calculate best-fit survival curves and the time of exposure at a given temperature lethal to 50% of the animals ($LD_{50/24\,hr}$) was determined [23].

RESULTS

The rise in temperature of rats heated to maximum temperatures, 41.5, 42.0 and 42.5°C are shown in Fig. 2. Core temperature rose rapidly to reach the temperature of the waterbath. It was possible to keep the rats at the desired temperature ±0.2°C, although at the higher temperatures and, in some cases, at the longer exposure times the temperatures became less stable. At temperatures less than 42°C and times of less than 1 hr the rats recovered from anesthesia within 15–20 minutes. As temperatures and exposure times increased the range of response, from death during heating to quick recovery, became wider. A sign of impending death was the development of cyanosis of the feet and eyes and labored breathing. Most of the rats that died did so without regaining consciousness, but some recovered to a semiconscious state and died within 24 hr. There were few deaths after 24 hr, although this was not thoroughly studied for all heating temperatures. Many of the rats developed diarrhea during and within the first few hours after treatment. The temperature of the rats fell rapidly after they were moved to the warm box (Fig. 3). Despite drying them and maintaining them on a water-heated pad at 38–40°C, the core temperature dropped to 35–36°C. There was no difference in the duration or magnitude of this drop between rats heated to 41.75 and 42.5°C. The temperature rapidly returned to normal as the animals regained consciousness, within 3 hr if they were going to recover. A similar drop in temperature was recorded from the sham-heated rats maintained at 37.1°C, but they recovered consciousness within 15–30 min.

The rats anesthetized with pentobarbital fared much worse than those anesthetized with halothane when heated to 42°C. While 10 of the 12 (83%) heated to 41.75°C for 1 hr survived

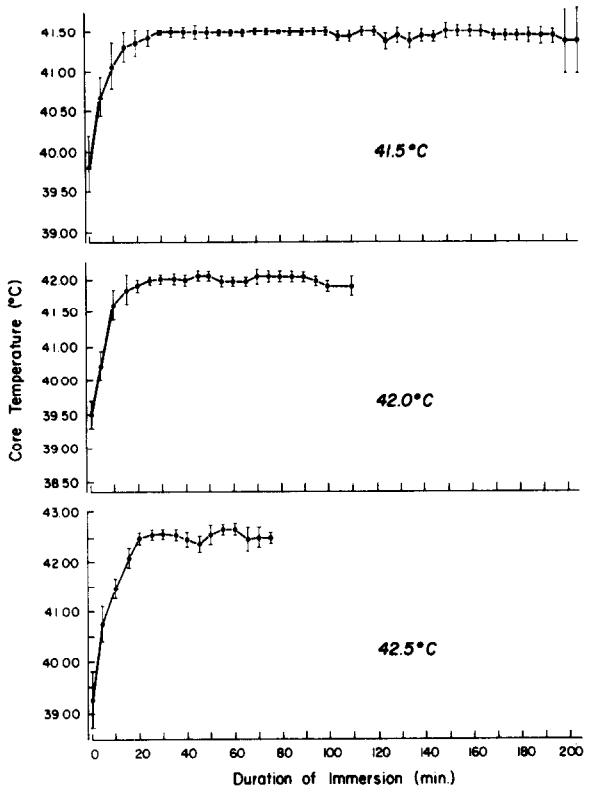

Fig. 2. Time-temperature curves for groups of rats heated to 41.5, 42.0 and 45.2°C. The error bars indicate 2 standard deviations about the mean for each data point, with at least 10 rats for each data point.

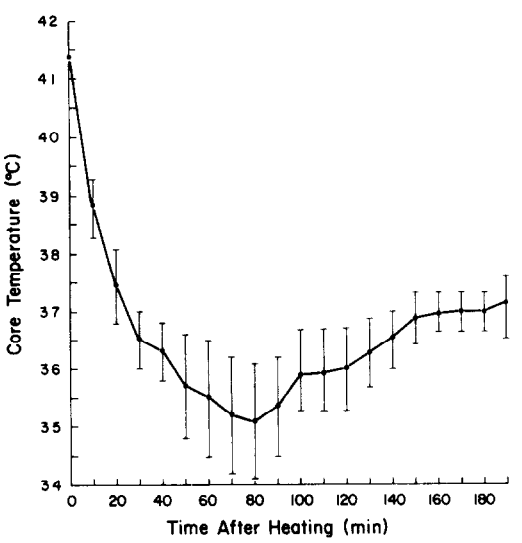

Fig. 3. Time-temperature curve obtained during recovery in a warm box of 8 rats anesthetized with halothane following their initial exposure to 41.75°C for 1 hr (±2 S.D. about the mean).

(compared with 90.4% of gas-anesthetized rats) their recovery period from the treatment was prolonged to 4–8 hr. None of the 12 rats heated for 25 min at 42.5°C recovered from anesthesia, and all were dead within 24 hr. The difference between the survival of this group and the survival of halothane-anesthetized rats exposed for the same time and temperature (54%) was highly significant ($P = 0.0001$), as determined by the chi-square likelihood ratio.

The rats anesthetized with halothane tolerated temperatures up to 41.75°C well, with almost 100% survival at that temperature for 1 hr, and 100% surviving exposures of 41.5°C for 3 hr. At higher temperatures mortality increased rapidly, with an $LD_{50/24\,hr}$ at 42.0°C of 60 min, and progressively shorter $LD_{50/24\,hr}$ at higher temperatures (Figs 4 and 5). The survival of rats was not influenced by the administration of i.p. Ringer's lactate solution (results not shown).

DISCUSSION

The results show that there is no inherent physiologic obstacle to obtaining useful survival curves following systemic hyperthermia in the rat at core temperatures of 41.5–42.75°C. We attribute these results to the use of gas anesthesia permitting rapid recovery and the careful post-hyperthermic management during the rat's recovery from the poikilothermic period following anesthesia and hyperthermia.

There are several disadvantages of pentobarbital as an anesthetic for rodents in radiotherapy research [24]. Because a single intraperitoneal injection is given, the duration and depth of anesthesia cannot be controlled. The margin of safety between anesthesia and respiratory arrest is low, heart function is depressed [24, 25] and pentobarbital anesthesia may decrease radiosensitivity, probably by inducing hypoxia [24]. This would be a disadvantage when systemic hyperthermia and radiotherapy are done on tumor-bearing animals. Barbiturates must be detoxified and excreted by the liver, which prolongs recovery, and anesthetic hypothermia is a problem [26]. These deleterious effects were demonstrated in our experiments using pentobarbital as the anesthetic agent, in which the recovery time from anesthesia was much longer than from halothane and oxygen, and the survival significantly lower at high temperatures (42.5°C).

Dickson and Ellis [13] reported only a 9.6% survival (16 of 167) for rats whose rectal temperature reached 41.5°C or more for 60 min under pentobarbital anesthesia. These were both normal rats and rats bearing a tumor on a hind leg, and they were heated by placing the hind leg in a hot water bath. The $LD_{50/24\,hr}$ at 41.6°C was 1 hr. We obtained 100% survival at 3 hr at 41.5°C, and our $LD_{50/24\,hr}$ at 41.75°C was 115 min (Fig. 4). Schechter *et al.* [15] also heated tumor-bearing hind legs of rats anesthetized with pentobarbital (25 mg/kg). The rats began to die at a core temperature of 40.7°C for 1 hr. We had almost 100% survival at 24 hr after a temperature of 41.75°C for 1 hr.

We limited our LD_{50} post-treatment survival time to 24 hr for two reasons: (1) very few animals that survived 24 hr died after this period; and (2) we wished to compare the $LD_{50/24\,hr}$ of these rats with that of other groups of rats treated similarly but conditioned 24 hr previously with sublethal heat exposure in a series of experiments on thermotolerance [23].

The depth of insertion of the thermoprobe is crucial to accurate measurement of core temperature. Lomax [27] and Schechter *et al.* [15] reported that a depth of 6 cm or more was necessary to

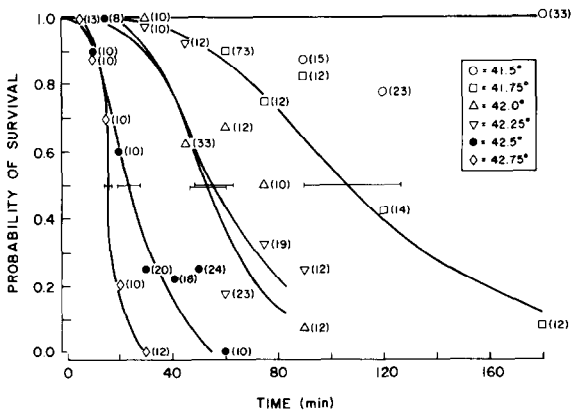

Fig. 4. Survival curves generated by linear logistic regression analysis from data points for times and temperatures as shown. The numbers in parentheses are the number of animals used for each determination. The error bars indicate ±2 S.D. for the heating time that killed 50% of the animals within 24 hr after treatment ($LD_{50/24\,hr}$).

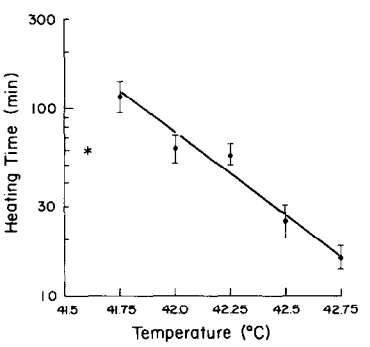

Fig. 5. Semilogarithmic plot showing exponential relationship between heating time and temperature to produce 50% mortality (error bars represent ±2 S.D.). The asterisk indicates the $LD_{50\,24\,hr}$ *of both normal and tumor-bearing rats anesthetized with pentobarbital and heated by placement of the hind leg placed in a waterbath* [13]. (*The* $LD_{50\,24\,hr}$ *at* 41.6°C *was approximately* 60 min. *A total of* 67 *rats were studied*).

determine a stable core temperature near the rat's liver. We also found that a lesser depth gave readings closer to the temperature of the waterbath as the probe was near the skin of the left side of the animal.

A system using light general anesthesia and immersion in a waterbath was considered to give the quickest and least stressful elevation of body temperature possible [28]. Heating a conscious animal in a box would be very stressful as the animals' thermoregulatory mechanisms would be severely taxed until overcome [28]. Experiments on resistance to heat stress have shown that once a conscious animal loses its ability to regulate its body temperature, an uncontrolled and fatal hyperthermia results [29–31]. Rats control their body temperature not by sweating or panting but by evaporative heat loss from saliva which they lick over their fur. General anesthesia abolishes these reflex actions. Rats also lose heat by radiation, convection and conduction from their hairless body surfaces, particularly the tail [32, 33]. For these reasons, rats do not become more than 8% dehydrated when subjected to heat stress for 8 hr [29]. This explains the lack of any beneficial effect of i.p. Ringer's lactate on survival.

By changing the degree of immersion of the tail and the whole body, it was easy to regulate the core temperature of the rats. The rats became temporarily poikilothermic after the heat treatment, and the body temperature dropped to 35–36°C before they recovered consciousness. Temporary poikilothermia, or loss of thermoregulation, is a phenomenon which follows severe heat stress [29, 31]. It is compounded by the abolition of thermoregulation by general anesthesia. Therefore, an anesthetic which allows rapid recovery is desirable. Halothane is a potent volatile anesthetic that is quickly eliminated in the expired gas, and has only a slight cardiovascular and respiratory depressant effect [34]. The latter is countered by giving oxygen as the carrier gas [24]. The pre-anesthetic sedative given, ketamine, is a dissociative general anesthetic agent with minimal cardiac depression [35] that has proven very safe in rodents and is far superior to barbiturates [35–37]. It was used only as a sedative dose to allow the mask to be held over the rat's head easily. A full anesthetic dose alone or with a tranquillizer given i.m. or i.p. would have prolonged recovery time [37], been uncontrollable and may have decreased survival. This concern is supported by the better survival results reported here than have been obtained by others who used ketamine and xylazine (Rompun): 42.0°C for 15 min whole-body hyperthermia was uniformly lethal [17].

The mechanism for heat-induced lethality remains unclear. The dramatic change in the slope of the survival curve with small temperature changes in the range of 41.75–42.75°C mirrors the steep change in cell killing noted both *in vitro* [38] and following local heating *in vivo* for the skin of mouse ear [39, 40] and for mouse intestine [41]. Law *et al.* reported that the time of heating for a 50% probability of necrosis of the skin of the mouse ear fell from approximately 375 min at 41.5°C to 90 min at 42.8°C [39, 40]. Similarly, Hume *et al.* [41] reported a decline on the D_0 (the reciprocal of the slope of the curve) for crypt loss following local heating of externalized small loops of mouse jejunum from 103 min at 42°C to 15.5 min at 42.5–42.8°C. The crypt loss following local heating occurred with a half-time of approximately 6 hr [41]. The rapid onset of diarrhea noted in some of our animals during heating suggests that gastrointestinal damage might be playing some role in causing death following whole-body hyperthermia. However, diarrhea was not noted in all the animals that died.

Histopathologic examination of the intestines of rats that died did not reveal crypt necrosis [Lord *et al.*, unpublished observations]. However, it does not rule out the possibility that the small intestine may be a target organ. Henle and Dethlefsen [19] suggested that heat may break down an intestinal barrier to bacterial infection and commented that the LD_{50} of Dickson and Ellis's rats [13] lay on the same curve as the LD_{50} points for mouse jejenum [41], indicating that intestinal damage may be the cause of death. However, we have demonstrated that this survival point is greatly influenced by the experimental conditions, particularly the method of anesthesia and post-hyperthermia care. Also, animals died during and shortly after heating, which suggests another, more quickly expressed damage to a target system or tissue. Additional studies will be needed to clarify any relationship between small bowel damage and lethality and to help elucidate other possible critical target tissues for killing by whole-body hyperthermia.

The method of heating described in this article permits a reproducible systemic hyperthermia in the rat at levels in the therapeutic range and can be utilized for experiments on fractionated heating, thermotolerance [23], tolerance to subsequent local heating [Weshler *et al.*, in preparation], thermochemotherapy and studies on the effects of systemic hyperthermia alone and with other modalities on tumors.

Acknowledgements—We thank Jennifer Morgen for her technical assistance and Roberta Lawrence and Diane Fischer for the statistical analysis.

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