American Journal of Hematology 64:262–270 (2000)

Alterations in Hemostasis Associated With Hyperthermia in a Canine Model

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Use of hyperthermia in the treatment of cancer and viral infection has received renewed interest. However, the in vivo relationship between hyperthermia and direct versus indirect effects upon hemostasis are incompletely defined, although we do know that disseminated intravascular coagulation (DIC) is a common sequel to heat stroke. The purpose of the present study was to more precisely define the relationship between hyperthermia and derangements of hemostasis, thereby providing a guideline for the development of safe hyperthermia treatment regimens. The present investigation examined the in vivo effects of high-grade whole-body hyperthermia (WBH) (42.5°C, 90 min) on hemostasis in a canine model. Induction of hyperthermia via extracorporeal circulation of heated blood (ECC-WBH) caused thrombocytopenia, increased plasma fibrin degradation products (FDPs), prolonged clotting times, increased serum liver enzymes, and evidence of spontaneous bleeding. However, when WBH was induced by peritoneal lavage (PL-WBH), transient thrombocytopenia was the only significant alteration. Temporal correlation between hemostatic alterations and elevations in serum alanine aminotransferase (ALT) levels in the ECC-WBH treatment group suggested that liver injury is responsible, at least in part, for the coagulopathy associated with high-grade hyperthermia and that in the absence of liver injury, identical degrees of hyperthermia cause only incidental decreases in platelet numbers. Am. J. Hematol. 64:262–270, 2000. © 2000 Wiley-Liss, Inc.

Key words: dog; hemostasis; hyperthermia; liver; thrombocytopenia

INTRODUCTION

Currently proposed therapeutic benefits of high-grade whole-body hyperthermia (WBH) include organ preconditioning against reperfusion injury and enhancement of anti-cancer and anti-viral therapies [1–3]. Despite the interest in these applications of controlled therapeutic hyperthermia, understanding of the in vivo relationship between hyperthermia and hemostasis remains limited despite known untoward effects of hyperthermia in clinical cases. Although heat stroke is associated with thrombocytopenia and/or disseminated intravascular coagulation (DIC) [4–6], the relationship between thermal dosage and alterations in hemostasis is unclear due to the variability of core body temperature in clinical cases. In clinical trials of induced therapeutic hyperthermia, treatment has been associated with tachycardia, respiratory alkalosis, and hepatocellular injury in addition to derangements of hemostasis [7–10]. However, these trials also do not adequately define hematologic effects of hyperthermia alone because they were conducted on cancer patients with advanced malignant disease and euthermic treatment controls were lacking.

Analysis of the hemostatic effects of hyperthermia has been further compromised by inadequate techniques for delivery of a uniform thermal dose. The use of heated water blankets, water-perfused suits, and hot wax baths to elevate core body temperature [7,8,10,11] in cancer patients was associated with excessive heating of skin [9]. Use of radiant heating devices has also been shown

Contract grant sponsor: National Institute of Neurological Disorders and Stroke; Contract grant numbers: R29 NS31693 and K04 NS01798; Contract grant sponsor: The State of Ohio Canine Research Fund.

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Received for publication 25 June 1999; Accepted 8 March 2000

to have a profound effect on temperatures at superficial sites that is not reflected by temperature measured at deeper sites [12], requiring insulation of extremities and/or humidification of anesthetic gases to improve temperature uniformity [13,14]. Exertion in a climatic chamber more closely achieves temperature uniformity and has been used in animal models of heat stroke [5,15]. However, thermal dosages remain variable in these systems since the experimental endpoints reflect either collapse and/or achievement of a target rectal temperature, where duration of that temperature endpoint (and thus the thermal dosage) is variable. An invasive method of regulated WBH should be most successful in achieving and maintaining uniformity of heating. One such method involves heating blood during extracorporeal circulation (ECC-WBH) [16–18]. However, the in vivo effects of this manipulation on hemostasis in healthy patients in a controlled setting are poorly characterized. The aim of this study was to examine potential in vivo alterations in general indices of hemostasis associated with a uniform hyperthermia. The canine model was selected for this study because clinical and hematological findings in experimental heat stroke in dogs parallel changes in humans as well as other mammalian species [5]. Heating of venous blood during extracorporeal circulation is an established technique for providing a uniform hyperthermia that is readily controlled [16,18], and the dog is an ideal model for manipulations involving extracorporeal circulation of blood due to the similarities between human and canine cardiovascular and pulmonary physiology. However, extracorporeal blood circuits and bypass systems have produced thrombocytopenia, functional platelet defects, consumption of coagulation factors and fibrinogen, and genesis of fibrin degradation products [19]. Therefore, peritoneal lavage of heated fluid was developed and used in this study to induce a uniform hyperthermia without direct manipulation of blood, thereby serving as a control for the nonspecific treatment effects on hemostasis that may accompany ECC-WBH. Although peritoneal lavage has shown promise as a safe and reliable method of providing regional hyperthermia in conjunction with intraperitoneal chemotherapy in clinical trials [20,21], its efficacy as a method to induce WBH (i.e., PL-WBH) has, until now, been unproven. The target thermal dosage of 42.5°C for 90 min was selected for this study, being clinically relevant both in proposed therapeutic regimens and in severe cases of heat stroke.

MATERIALS AND METHODS

Extracorporeal Circulation WBH

Ten adult (30 kg) male and female dogs underwent extracorporeal circulation of venous blood. Hyperthermia (42.5°C, 90 min) was induced in 6 dogs (ECC-WBH); 4 dogs underwent extracorporeal circulation without WBH as euthermic perfusion controls. Protocols used were approved by the Institutional Laboratory Animal Care and Use Committee. Dogs were tranquilized with Acepromazine (0.55 mg/kg) and atropine (0.04 mg/kg) and anesthetized with sodium pentobarbital (25 mg/kg induction and 6 mg/kg/hr maintenance). Electrocardiograms were monitored, and the dogs were artificially ventilated following paralysis with pancuronium bromide (0.1 mg/kg). The left and right femoral veins were cannulated and connected to the extracorporeal circulatory unit, a prototype heating device developed by *iP Scientific* (St. Louis Park, MN). Blood was withdrawn from the caudal vena cava through one femoral vein using a double-occlusive roller pump, passed through a heat-exchanger, and returned to the contralateral femoral vein. Heparin was administered intravenously throughout perfusion at dosages sufficient to maintain an activated clotting time of greater than 200 sec (11,000 units average cumulative dose). Arterial blood gases, blood pH, and systemic arterial blood pressure were monitored through a cannula in the left femoral artery. Percutaneous cannulation of the right external jugular vein was used to introduce a Swan-Ganz monitoring catheter and thermistor. The temperatures of tympanic membranes, deep rectum, bladder, esophagus, subcutaneous tissues, pulmonary arterial blood, and afferent and efferent venous blood (relative to the pump) were monitored continuously using individual thermistors placed at each recording site which, in turn, relayed these readings to a microprocessor built into the circulatory unit (Fig. 1). The microprocessor-controlled rate of blood heating and pump flow rate to assure temperature uniformity within the body to within +0.5°C. Rectal temperatures were measured at 8, 16, and 24 hr postperfusion and daily thereafter.

Peritoneal Lavage WBH

Nine adult male and female dogs underwent peritoneal lavage. Hyperthermia (42.5°C, 90 min) was induced in 6 dogs (PL-WBH), and 3 dogs received peritoneal lavage without hyperthermia as euthermic lavage controls. Induction and maintenance of anesthesia were as described for dogs undergoing extracorporeal circulation of blood.

Electrocardiograms, arterial blood gases, blood pH, and systemic arterial blood pressure were monitored, and animals were paralyzed and artificially ventilated. The arterial and venous infusion lines of these dogs were primed and flushed at the rate of 1 ml/hr with heparinized saline (1000 units/l, 5 units average cumulative dose). Peritoneal cannulae were introduced via a ventral abdominal midline incision. One cannula was advanced to the lower left quadrant of the peritoneal cavity and served as the entry site for circulating Plasmalyte® (Baxter, Deerfield, IL), while the other was positioned in the up-

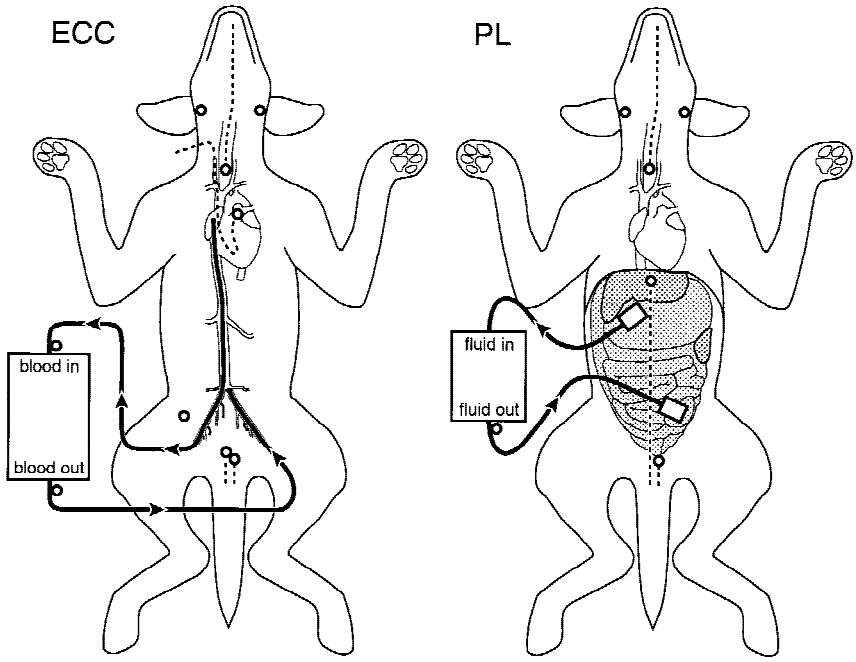

Fig. 1. Extracorporeal circulation (ECC) and peritoneal lavage (PL) systems used to induce whole-body hyperthermia (WBH) in dogs. The schematics illustrate dogs in dorsal recumbency. The pump and heating apparatus are shown as a box with the flow circuit indicated by arrows. Open circles indicate thermistor placement. See Materials and Methods for description.

per right quadrant for withdrawal of abdominal fluid and recirculation. The abdominal cavity and pump circuit were primed with 2 L of Plasmalyte®. The circuit was driven by a centrifugal pump and flow rate manually regulated based upon readings from flow sensors. The temperatures of tympanic membranes, rectum, esophagus, efferent fluid (relative to the pump), and liver were monitored using individual thermistors placed at each recording site (Fig. 1). In two of the PL-WBH dogs, an additional thermistor directly measured the temperature of the cerebral cortex. The heating device was as described for ECC-WBH, and temperature uniformity was monitored and maintained to within ±0.5°C. Rectal temperatures were measured at 8, 16, and 24 hr post-lavage.

Indices of Hemostasis

For the ECC-WBH experimental group, serial blood samples were collected 24 hr pre-ECC, at the end of the 90-min hyperthermic interval (time 0), and at 8, 16, 24, 72 hr, and 8 days post-perfusion. In the PL-WBH experimental group, blood was collected at 24 hr pre-treatment, t_0, and 8, 16, and 24 hr post-lavage. At each time point, serum biochemical parameters were evaluated with the Hitachi 911-Automatic Analyzer® (Boehringer-Mannheim, Indianapolis, IN). Hemograms from EDTA-chelated whole blood were also performed at each time point using the Coulter Counter® Model S-Plus IV Hematology Analyzer (Coulter Electronics Inc., Hialeah FL). Platelet counts less than 50,000/μl were generated manually. Citrated plasma was used to establish general indices of coagulation, including whole-blood clotting times and fibrinogen concentration. Single-stage prothrombin and activated partial thromboplastin times (OSPT and APTT) were measured using a centrifugal coagulation analyzer (Instrumentation Laboratory ACL 200®, Instrumentation Laboratory Company, Lexington MA). Fibrinogen concentration was measured using the Instrumentation Laboratory ACL 200. The presence of FDPs was detected in samples treated with thrombin soybean trypsin by the Thrombo-Wellcotest® (Murex Biotech Ltd, Dartford, England) latex bead agglutination test. Plasma samples were considered positive for FDPs if agglutination was noted in a 1:20 sample dilution. The serum biochemistry panel, hemogram, and coagulation tests were performed by The Ohio State University Veterinary Teaching Hospital hematology laboratory under Good Laboratory Practice (GLP) conditions. Statistical analysis of data was conducted using the Mann–Whitney nonparametric test for unpaired data.

RESULTS

For ECC-WBH dogs, core body temperature was increased at the rate of 0.1°C/min at a pump flow rate of 0.4 l/min and the target core maintained at 42.5 ± 0.5°C for 90 min (i.e., an average of all thermistor recordings except those measuring blood entering and exiting the heating device) (Fig. 2). During the heating interval between normal body and target temperature (i.e., ramp-up), recordings from all individual thermistors varied less than 1°C from the core average with the exception of blood leaving the heating device. For the latter, temperatures never exceeded the target by more than 1°C and never exceeded the core average by more than 4°C.

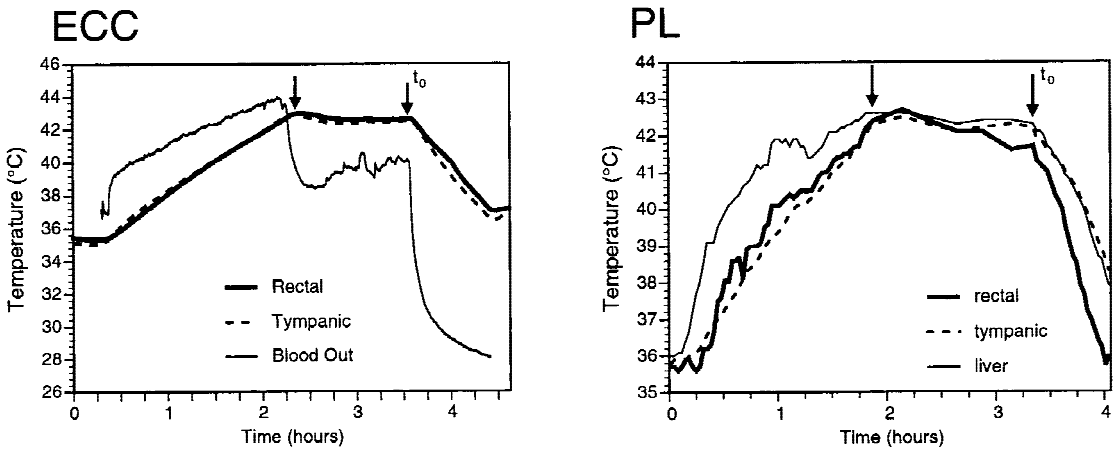

Fig. 2. Representative temperature curves from anesthetized dogs undergoing ECC-WBH and PL-WBH. For the ECC-WBH dog, recordings taken every second from the rectal and tympanic thermistors are presented, being representative of the average measurements from the tympanic membranes, esophagus, pulmonary arterial blood, rectum, bladder, and subcutis. These recordings are contrasted to blood leaving the heating apparatus. For the PL-WBH dog, recordings taken every 2 min from the rectal and tympanic thermistors are compared to liver temperature. An initial hypothermia was associated with anesthetization; normal canine body temperature is between 37 and 38°C. Arrows delimit the 90-min interval that the animals were maintained at target temperature (average of 42.5 ± 0.5°C for all thermistors except those monitoring fluids leaving the pump) and the end of that interval was designated time 0 (t_0).

Throughout the 90-min interval at target temperature, recordings from individual thermistors never exceeded the core average by more than 0.5°C, with the exception of cooler temperatures for blood exiting the heating apparatus. For PL-WBH, core body temperature was increased at a rate equivalent to ECC-WBH and was similarly maintained at the target level of 42.5 ± 0.5°C for 90 min (Fig. 2). In light of the correlation between pulmonary arterial blood temperature and the core average in the ECC-WBH dogs, the Swan-Ganz thermistor/catheter was not used during peritoneal lavage in order to minimize invasiveness of the procedure. During the ramp-up to target, rectal and liver temperatures varied in response to changes in temperature of lavaged Plasmalyte®, although fluctuations from core average did not exceed 1.0°C. Once target temperature was achieved, recordings from individual thermistors, including rectal and liver, varied less than 0.5°C from the core average during the subsequent 90-min interval. For both ECC- and PL-WBH, core temperature was returned to 37°C within 30–45 min by continuing either ECC or PL without heating. Euthermic treatment controls were perfused or lavaged in an identical manner and duration as their heated counterparts, but body temperature was maintained at 37 ± 0.5°C. Recovery from anesthesia and ECC or PL was uneventful, and rectal temperatures during the post-hyperthermic interval revealed normal body temperature regulation in both heated and control groups.

Reference ranges for indices of hemostasis, hemograms, and serum chemistry profiles were separately established for the extracorporeal circulation and peritoneal lavage treatment groups using values obtained from blood samples collected 24 hr prior to treatment for all dogs enrolled in the study. With the ECC system, euthermic controls experienced a 40% decrease in platelet count by t_0, the end of the target hyperthermia interval (Fig. 3). In unheated controls, nadir was 8 hr post-perfusion, representing the only significant ($P = 0.049$) decrease from the pretreatment reference range. Dogs undergoing ECC-WBH demonstrated a significant decrease in platelet numbers relative to both the reference range and values obtained from the euthermic controls for all time points through 3 days post-hyperthermia. Platelets decreased to less than 20,000 platelets/μl at 16 and 24 hr post-treatment, with nadir at 24 hr. Spontaneous bleeding was observed in all dogs at 16 and 24 hr post-treatment and was demonstrated most consistently as blood in the stool, with only sporadic epistaxis. Such bleeding was not observed after 24 hr post-hyperthermia when platelet numbers exceeded 20,000/μl. Platelet counts returned to within the reference range by 8 days post-treatment. Dogs undergoing PL-WBH demonstrated a significant decrease in platelet numbers relative to both the reference range and values obtained from the euthermic control dogs from t_0 through 24 hr post-t_0, as in ECC-WBH, although counts did not continue to drop after 8 hr post-treatment and remained greater than 20,000/μl. Spontaneous bleeding was not observed in the PL-WBH treatment group. The effects of peritoneal lavage alone on platelet numbers were minimal and transient, with the average decrease in platelet counts being only 30% at t_0 in euthermic PL controls. This decrease, although statistically significant ($P = 0.01$), was associated with a mean platelet count that was equivalent to

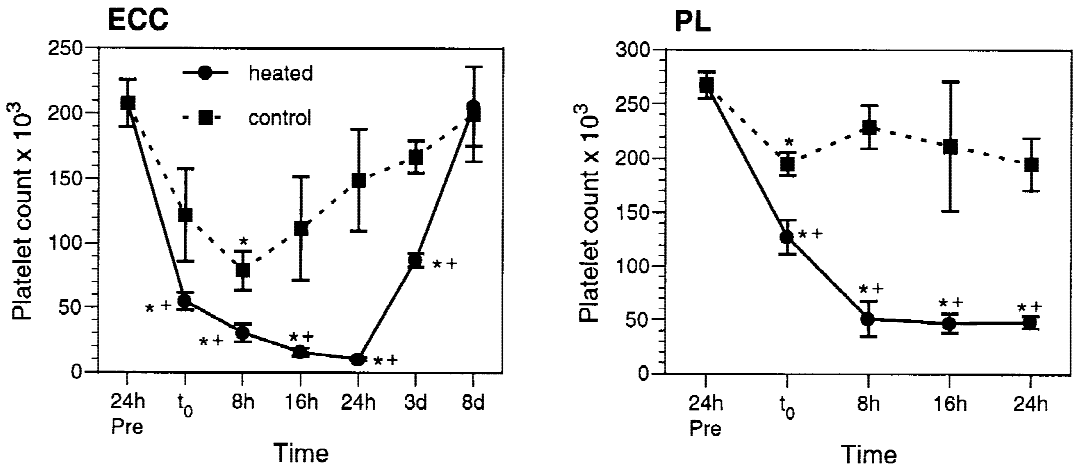

Fig. 3. Effect of ECC and PL whole body hyperthermia on platelet counts. Sample variances indicate the standard error of the mean. The pretreatment reference range was calculated based upon values derived from the animal population under study for each induction method. Significant deviation ($P < 0.05$) from the reference range (*) or significant difference between heated and euthermic control values (+) was calculated using the Mann–Whitney comparison of non-parametric distributions.

mean counts from later time points (i.e., 24 hr post-t_0) that were not considered to be outside of the reference range based upon statistical analysis.

Fibrinolysis was associated with ECC as demonstrated by increased fibrin degradation products in plasma. FDPs were detected in 1:20 sample dilutions from both ECC-WBH and euthermic perfusion control dogs at t_0 and 16 and 24 hr post-perfusion. At t_0, five of six heated dogs and three of four nonheated dogs had FDPs in their plasma. At 16 hr post-ECC, three of six heated and three of four control dogs were positive for FDPs. At 24 hr post-ECC, four of six heated and one of four control animals had FDPs in their plasma. There was no evidence of fibrinolysis in the peritoneal lavage system based upon the absence of FDPs in 1:5 sample dilutions at all time points. Reductions in fibrinogen, a characteristic feature of DIC, were not observed in either the ECC or PL system (Fig. 4). Conversely, fibrinogen concentration increased significantly in all dogs, consistent with the induction of fibrinogen as an acute phase reactant.

Dogs undergoing ECC-WBH showed significant prolongation of both OSPT and APTT at t_0, 8, 16, and 24 hr post-treatment, with values returning to the normal range by 3 days post-treatment (Fig. 5). The 10-fold increases in APTT at t_0 in both ECC-WBH and perfusion controls was attributed to systemic administration of heparin and/or transient factor XII depletion caused by contact with the extracorporeal circuit [19] and induction of a systemic stress response [22]. Effects of heparin were reversed by administration of protamine sulfate (0.8 mg/kg) at the end of the perfusion interval, permitting detection of ECC or ECC-WBH induced changes in APTT at later time points. ECC alone was without statistically significant effect on clotting times at other time points. With PL-WBH, statistically significant prolongation of OSPT was observed at t_0, 8, and 16 hr post-treatment, although the prolongation did not exceed the reference range average value by more than 1 sec. APTT in these dogs was statistically significantly prolonged at t_0, and 8 hr post-hyperthermia; however, the magnitude of these delays did not exceed the reference range by more than 15 sec. Peritoneal lavage alone was not associated with prolongation of clotting times; the less than 10-sec prolongation of APTT at t_0 in PL euthermic control dogs reflects the administration of heparin to maintain patency of the arterial and venous infusion lines in these dogs.

The hemograms of venous perfusion and peritoneal lavage dogs, both heated and control, were unaltered by treatment. Normal red blood cell parameters were observed and no RBC fragments were noted on blood smears. Likewise, serum biochemical profiles of all dogs were normal at all time points except for liver enzymes. Increases in concentration of the enzyme alanine aminotransferase (ALT) were noted in ECC-WBH dogs at 8, 16, 24, 72 hr, and 8 days post-hyperthermia (Fig. 6). Maximal increases were approximately 30-fold over reference range values at 16, 24, and 72 hr after ECC-WBH, followed by a return toward the reference range by 8 days post-hyperthermia. Changes in ALT concentrations in control dogs were minimal with ALT concentration exceeding the reference range by only 5–20 IU/l at 3 and 8 days post-t_0. Aspartate aminotransferase levels in ECC-WBH demonstrated similar increases to those of ALT, with peak increases nearly 80-fold over reference values at 16 hr post-hyperthermia and a return toward reference range by 3 days post-t_0 (data not shown). The magnitude of ALT elevations was correlated with the degree of structural hepatocellular injury documented by histologic examination. The latter revealed moderate hepatocellular

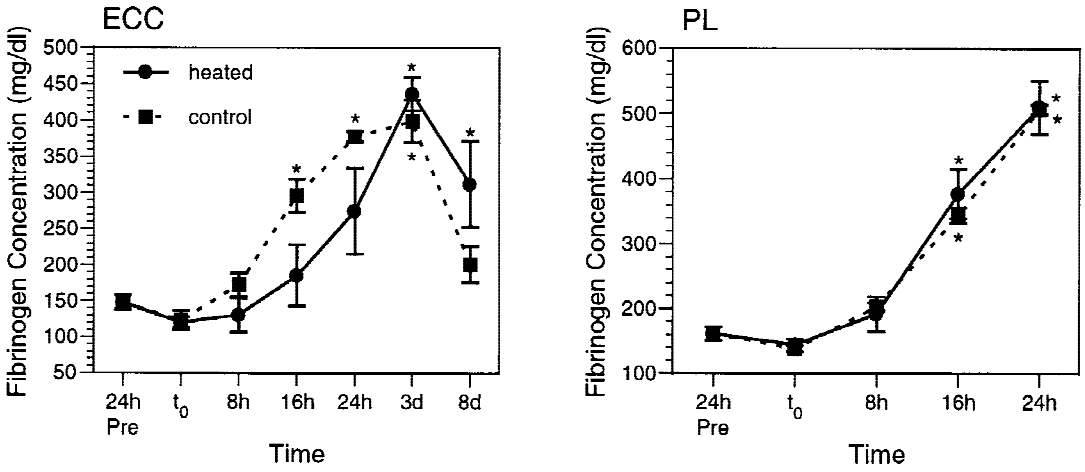

Fig. 4. Effect of ECC and PL whole body hyperthermia on plasma fibrinogen concentration. Sample variances indicate the standard error of the mean. The pretreatment reference range was calculated based upon values derived from the animal population under study for each induction method. Significant deviation ($P < 0.05$) from the reference range (*) or significant difference between heated and euthermic control values (+) was calculated using the Mann–Whitney comparison of non-parametric distributions.

vacuolar change with a centrilobular and midzonal distribution at 8 days post-hyperthermia. Under peritoneal lavage, there was no significant difference between ALT concentrations of heated and control dogs. Both heated and euthermic control PL animals had statistically significant increases in ALT concentration at 8, 16, and 24 hr post-lavage (data not shown). However, the rise in ALT did not exceed the reference range by more than 2-fold and histologic changes in liver were not observed. Again, aspartate aminotransferase levels paralleled those of ALT.

DISCUSSION

Disseminated intravascular coagulation is a consumptive coagulopathy defined by thrombocytopenia, prolonged clotting times (OSPT and APTT), the presence of fibrin degradation products in plasma, decreased fibrinogen concentration, red blood cell fragments in circulation, and/or spontaneous bleeding. Historically, both heat stroke and therapeutic hyperthermia have been associated with DIC [4–6,11]. In the present study we show that ECC-WBH is associated with a transient coagulopathy having characteristics of DIC, including thrombocytopenia, prolonged clotting times, spontaneous bleeding, and presence of FDPs. However, ECC alone caused thrombocytopenia and generation of fibrin degradation products. These results are consistent with published work showing that ECC of blood can cause platelet activation and sequestration as well as fibrinolysis [19,23]. The fact that ECC-WBH resulted in qualitatively similar changes, but of a greater magnitude, indicates that the effects of hyperthermia on hemostasis are additive to the effects of ECC alone. In particular, in vitro studies have shown that hyperthermia also induces platelet aggregation [15]. Unexplained, however, is the prolongation of clotting times in ECC-WBH, particularly in light of results whereby the in vivo effects of high-grade hyperthermia induced by PL can be restricted to a transient suppression of platelet counts.

The difference between the selective decrease in platelet number in PL-WBH and the protracted and more profound reduction in platelet numbers in ECC-WBH, together with evidence of fibrinolysis and prolonged clotting times in the latter, may be explained by an effect of ECC on liver function. Liver injury has been documented in ECC, where that injury reflects the effects of hypoperfusion, microembolism, and damage associated with oxygen free radicals [24]. Furthermore, previous studies demonstrated that ECC-induced hepatocellular injury is exacerbated by increased temperature and that circulatory manipulation actually lowers the temperature threshold for thermally induced liver damage [25–27]. The additive effects of hyperthermia are indicated in the present study by dramatic increases in ALT over euthermic control values and presence of histologic liver changes characterized by hepatocellular vacuolization in the ECC-WBH experimental group. These histologic results are consistent with previous investigations where centrilobular hepatocellular vacuolar change was induced by hyperthermia, with changes progressing to ballooning degeneration and necrosis [25,28]. The liver injury in ECC-WBH does not likely reflect excessive heating despite the fact that liver temperatures were not specifically measured. Blood introduced from the heating device transiently exceeded the target temperature by 1°C prior to achieving the core average target temperature but these fluids bypassed the liver since they were

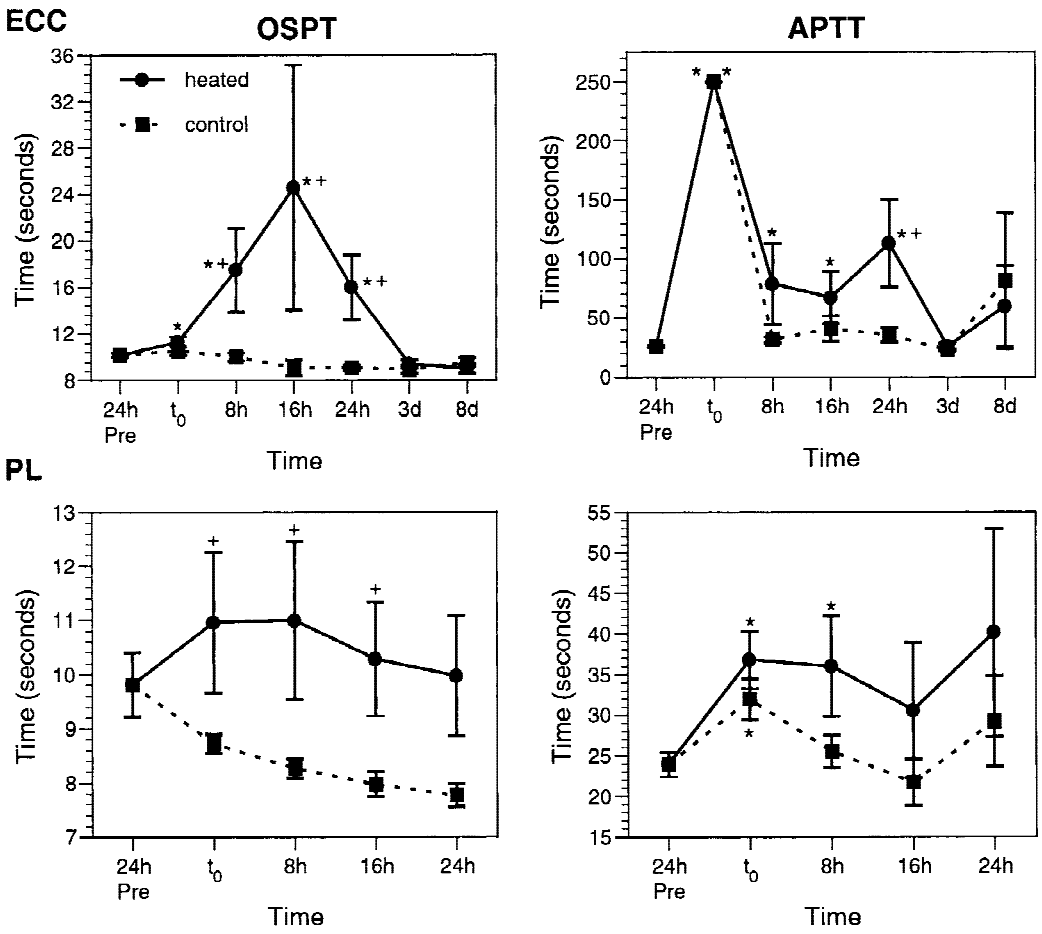

Fig. 5. Effect of ECC and PL whole-body hyperthermia on one-stage prothrombin and activated partial thromboplastin clotting times (OSPT and APTT, respectively). Sample variances indicate the standard error of the mean. The pretreatment reference range was calculated based upon values derived from the animal population under study for each induction method. Significant prolongation ($P < 0.05$) from the reference range (*) or significant difference between heated and euthermic control values (+) was calculated using the Mann–Whitney comparison of non-parametric distributions.

directly introduced into the femoral vein/caudal vena cava; pulmonary arterial blood temperatures were consistently within 0.5°C of the core average.

Enhanced liver injury in ECC-WBH can explain the biphasic release of FDPs. The initial fibrinolysis can be attributed to a direct effect of ECC, consistent with previous work showing that ECC can induce primary fibrinolysis [19,29]. The second appearance of FDPs at 16–24 hr post-treatment is correlated to the point of maximal hepatocellular injury indicated by serum ALT levels, consistent with the established relationship between liver injury and fibrinolysis [30]. Furthermore, the augmented liver injury in ECC-WBH can explain the more severe reduction in platelet counts at 16–24 hr post-treatment since hepatocellular injury can cause thrombocytopenia [30,31]. At these time points, platelet counts drop below 20,000/μl and spontaneous bleeding occurs. The prolonged clotting times observed at 8–24 hr post t_0 with ECC-WBH may also be explained by enhanced liver damage in ECC-WBH. Augmented liver injury in ECC-WBH could compound depletion of clotting factors caused by ECC alone while maintaining the ability to produce acute phase reactants such as fibrinogen [22].

The lack of hemostatic complications in PL-WBH, when using the same thermal dosage employed in the ECC-WBH treatment group, is correlated to lack of evidence of hepatocellular injury in the former. The absence of hepatocellular injury was based upon failure of serum ALT levels in the PL-WBH group to rise above those observed in the euthermic PL controls. Serum ALT concentrations were mildly increased at 8, 16, and 24 hr post-treatment in both heated and control animals, but these increases likely reflect an anesthetic effect as sodium pentobarbital is metabolized by the liver. Furthermore, hepatocellular vacuolar change was not observed following histologic examination of the liver at 24 hr post t_0, a time when vacuolar change was evident in dogs from an ECC-WBH pilot study (unpublished data, 1998). Absence of hepatocellular injury in PL-WBH was attributed to the minimal invasiveness of the procedure; he-

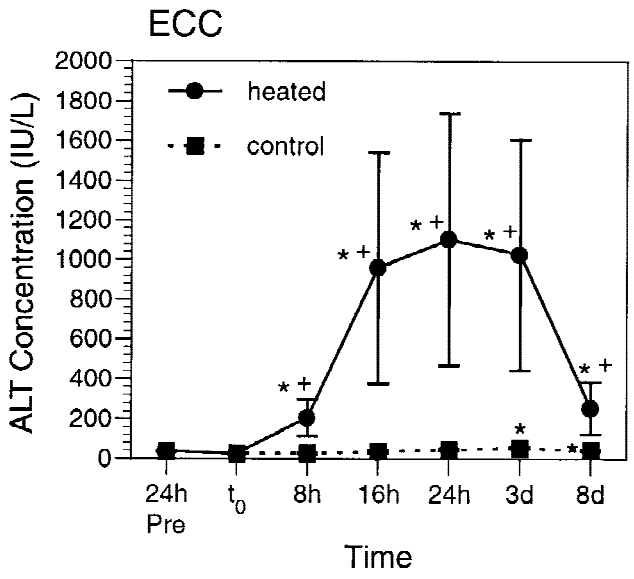

Fig. 6. Effect of ECC whole body hyperthermia on serum alanine aminotransferase (ALT) concentration. Sample variances indicate the standard error of the mean. The pretreatment reference range was calculated based upon values derived from the animal population under study for each induction method. Significant increase ($P < 0.05$) from the reference range (*) or significant difference between heated and euthermic control values (+) was calculated using the Mann–Whitney comparison of non-parametric distributions. There was no difference between rises in ALT concentration in heated and control dogs in PL-WBH (data not shown).

matologic changes reflect only the direct effects of hyperthermia without additional alterations associated with the WBH induction procedure. Taken together, the correlation between the kinetics of elevations in liver enzymes and the DIC episode in ECC-WBH and lack of hepatocellular injury and DIC in PL-WBH supports hepatic involvement as a key determinant of the response to a single high thermal dosage (i.e., selective thrombocytopenia or DIC).

Other adverse effects of hyperthermia reported in heat stroke may reflect physiologic responses to increased temperatures that may include tachycardia and respiratory alkalosis. Tachycardia may result in decreased cardiac output and respiratory alkalosis associated with hyperventilation represent potentially life-threatening complications in the dog (unpublished data, 1998). However, both of these responses can be controlled, as in the present study, using β adrenergic receptor antagonists to regulate heart rate and paralysis with mechanical ventilation to prevent hyperventilation, thereby permitting the isolation of the in vivo hematologic effects of hyperthermia alone. Data presented here have implications for both understanding the pathogenesis of heat stroke and the judicious and responsible use of hyperthermic treatment regimens. Further investigation is warranted to identify the determinants of hepatocellular injury at temperatures less than 43°C that dictate the progression of hemostatic derangements towards DIC versus thrombocytopenia alone.

ACKNOWLEDGMENTS

This work was supported by funds from the National Institute of Neurological Disorders and Stroke (R29 NS31693 and K04 NS01798) and The State of Ohio Canine Research Fund. Instrumentation for WBH was provided by *iP Scientific*. The authors thank Deborah Frolicher of The Ohio State University for invaluable technical assistance.

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