Original Article

Whole Body Extracorporeal Low Flow Hyperthermia in a Canine Model

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ABSTRACT

Whole body hyperthermia (WBH) has not gained significant clinical acceptance, though extensive experimentation since the early 1980's has revealed its potential merits in the treatment of various cancers, and currently WBH is in clinical feasibility trials for Acquired Immune Deficiency Syndrome (AIDS). Using a new device and methodology, could canines serve as an appropriate test model for this device and methodology? Five dogs underwent one treatment each of the 42°C whole body hyperthermia using a low flow veno-venous approach. All animals were kept at the desired temperature for 90 minutes. There were no clinically significant acute or long term sequlae. Every animal was subjected to necropsy. Findings of this study showed that the canine was an adequate model to assess the initial safety of this new device.

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INTRODUCTION

Whole body hyperthermia (WBH) is not novel, and non-invasive methods in inducing hyperthermia have revealed potential benefit in the field of oncology. Various clinical protocols are actively exploring cancer therapy at leading academic centers in this country, as well as abroad. Both invasive and noninvasive methods are being studied as possible treatment options for Acquired Immune Deficiency Syndrome (AIDS). Governmental approval as a potential treatment has been lacking to date; however, clinical results have reported remarkable success in the treatment of malignant lesions.

Methods of inducing WBH have mainly focused on non-invasive means, such as microwaves, ultrasound, paraffin wax baths, high temperature hydrotherapy, hot water blankets, and "radiant energy cocoon" devices (1). Each method carries its own risks during treatment; however, most researchers and clinicians consider non-invasive techniques to be within acceptable limits. This has not been the case with an invasive approach. Past experience using extracorporeal methods have been shown to carry higher risks (2); however, with new technology and methods of treatment management, these risks have decreased. An extracorporeal method using a hemodialyzer modified from Parks and Smith has been recently reported for WBH in humans (3). We have designed and developed a non-hemodialyzing whole body extracorporeal hyperthermia (EWBH) method which may carry even lower risks than the device used by Weidemann, et al. The premise of this study was to use canines to assess the appropriateness of the model in testing of this new device and methodology.

MATERIALS AND METHODS

All animals were procured and research performed according to all state and federal guidelines for the use of animals. The protocol had approval from the animal care and use committee at an accredited facility. In addition, the protocol was performed and monitored according to Food and Drug Administration Good Laboratory Practice guidelines.

Laboratory raised male dogs, weighing between 27.6–33.8 kg, were kept NPO the evening prior to surgery. A perioperative antibiotic, cefadroxil monohydrate (22mg/kg), was given to each animal. The evening prior to surgery, each animal received dexamethasone (1 mg) intramuscularly. On the morning of surgery, each animal was given an additional dose of dexamethasone (1 mg, intravascular), after intravenous access was established. Intravenous (IV) fluid replacement (D5W) was initiated at a rate of 75-100 ml/hr prior to the beginning of heating. The animal was positioned in a supine position on the operating table, prepped, and draped sterilely. The animal was anesthetized using sodium pentothal 2.5% and ventilated with a respirator[a] with supplemental oxygen to keep $pO_2 > 100$ mmHg. Intraoperative anesthesia was maintained with isoflurane 1.5-2.0%. Electrocardiac limb leads were placed for continuous electrocardiographic assessment. A foley catheter was inserted to assess urine output.

Surgical cut downs involving the left and right lateral cervical areas, and both left and right femoral areas were performed. Both external jugular veins were isolated in each cervical incision, and the left femoral vein and right femoral artery were isolated in each respective incision. An arterial (Tygon tubing) catheter was positioned in the right femoral artery for arterial pressure monitoring and for blood sampling. A 5 Fr Swan-Ganz catheter[b] was positioned properly in the main pulmonary artery. Multiple cardiac outputs prior to cannulation were obtained. Esophageal, rectal, and external jugular vein effluent temperatures were monitored continuously with thermistor probes[c]. Perfusion temperature was monitored with a Tuohy-Borst thermistor probe[c]. Main pulmonary blood temperature was monitored continuously via a thermistor in the indwelling Swan-Ganz catheter. Temperatures measured at each probe site and the heat exchanger's water bath were continuously displayed and recorded at five minute intervals. A cardiac output monitor[d] was used to measure cardiac output and pulmonary artery temperature. Digital read-out of the remaining temperature sites were obtained from a temperature device[b] and the PS-1 console panel[e]. All temperature equipment and probes were calibrated prior to initiation of the protocol. A typical temperature graph is shown in Figure 1.

Anticoagulation was satisfied with heparin (150 u/kg) and the animal was placed on veno-venous extracorporeal circulation using the PS-1 centrifugal perfusion system[e]. Activated clotting times (ACT) were measured to assess an anticoagulation state of at least one and one-half of normal. Following the initial dose of heparin, additional doses were given as needed to regulate ACTs. Straight cannulae were placed, one into the inferior vena cava via the left femoral vein (18-21 Fr)[f] and another was placed into the right atrium via the right external jugular vein (18-21 Fr)[f]. Efferent flow was from the left femoral vein, and inflow from the perfusion circuit was directed into the right atrium via the indwelling cannula. A low flow approach was used on extracorporeal bypass, which ranged between 10-15% of the animal's baseline cardiac output.

The perfusion circuit was primed with Plasmalyte A[g] (280 ml). Blood was not used as part of the priming solution, but would have been if the preoperative hemoglobin was below 10 mg/dl. No blood replacement was given to the animal or added to the pump during the procedure. Fluid addition with Plasmalyte

a Harvard Model #613, Harvard Insitute, South Natick, MA
b Spectromed flow directed thermodilution catheter, SP 510H, Baxter Laboratories, Irvine, CA
c Electromedics, Englewood, CO
d Model 9502A, Baxter Laboratories, Irvine, CA
e Organetics, Ltd. Minneapolis, MN
f Biomedics extracorporeal cannula, straight 50 cm tip, Medtronic Biomedicus, Eden Prairie, MN
g Baxter Healthcare, Deerfield, IL

A was supplied as needed during the procedure.

After beginning perfusion, the animal was heated gradually to reach the desired core temperature of at least 42.0°C (range 42.4-42.5°C) in > 40 minutes (Figure 1). The indicator temperature used to signal the beginning of plateau phase was either the esophageal or rectal temperature, whichever was higher. The animal was covered with a sheet to preserve heat. This warming phase ranged between 45-60 minutes, with a blood temperature range during this period of 45.2-48.0°C. The rate of IV replacement during the heating and plateau phases was increased to 150-200 ml/hr. Ice packs were placed at the back of the neck when a core temperature (either esophageal or rectal) of 40.0°C was reached.

Once the plateau phase was reached, each animal was maintained at plateau for 90 minutes. One to two doses of mannitol (10 mg) were given intravascularly during the plateau phase. At the completion of plateau, the cooling phase began and the sheet covering the animal was removed. The cooling phase occurred in two steps: passive and active. After 20 minutes of passive heat dissipation, a cooling coil in the afferent end of the heater/circulator was submerged in an ice water bath to actively cool the blood for an additional 25-35 minutes. Once animal core temperature fell to approximately 39°C, perfusion was suspended. All cannulae were removed, and the animals' heparinization was reversed with protamine sulfate, based upon values derived from the dose response curve (10 mg of protamine: 1000 units of heparin).

The right external jugular vein was ligated following removal of the cannula. A left femoral venoplasty was performed with the removal of its cannula. Additional cardiac outputs were performed prior to removal of the Swan-Ganz catheter . The left external jugular vein was ligated with removal of the Swan-Ganz catheter. The arterial catheter was removed with the arteriotomy repaired primarily. Anesthetic agents were discontinued, and all incisions were then closed in routine fashion. Animals surfaced from anesthesia within 0.5-1.0 hour. Each animal was kept in a holding cage for the first night following surgery and assessed hourly. Antibiotics were continued for seven additional days following the procedure.

All animals except one were sacrificed on day seven, and a formal post-mortem examination, including gross and microscopic sectioning, was performed. Due to a scheduling problem, the final animal was not sacrificed until day ten. Tissue samples of the following organs were obtained: brain (cerebral cortex, cerebellum), spinal cord, skeletal muscle samples, thymus, thyroid, parathyroids, lungs, heart, gastrointestinal tract, liver, spleen, adrenals, kidneys, bladder, pancreas, vena cava, aorta, and lymph nodes (regional and mesentery).

Figure 1: Typical heating curve of a canine undergoing EWBH with an Organetics PS-1

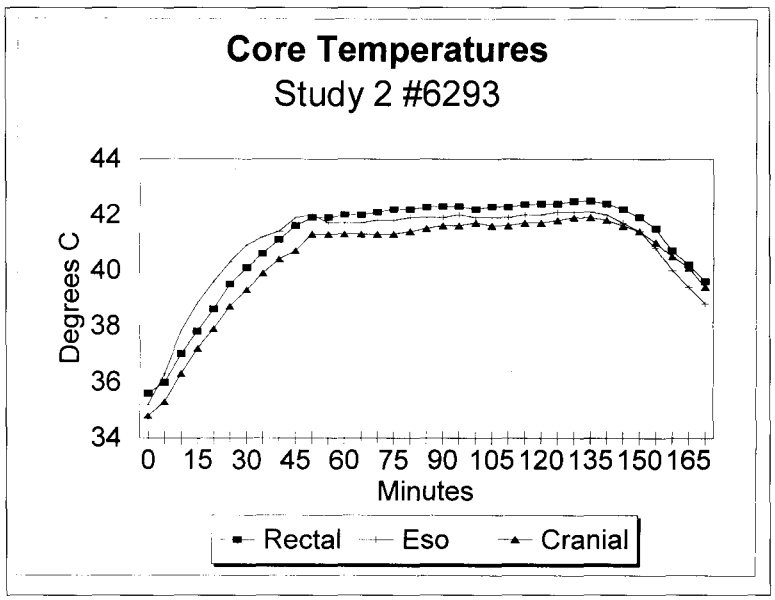

Table 1: Control equals one animal; values for heated animals (5) are represented at average ± standard deviation

CONTROL	T PROT	ALB	T BILI	AMY	Ca	PO4
Pre	6.2	3.4	0.1	658	10.2	4.6
P-END	5.3	2.9	0.2	507	9.4	7.4
Post Prot/COOL	5.1	2.9	0.2	511	9.0	6.6
Post Day 1	5.6	3.1	0.1	535	9.7	3.2
Post Day 4	6.1	3.3	0.3	726	10.1	5.2
Post Day 7	6.3	3.5	0.1	663	9.9	4.7
AVG ANIMALS 1 - 5 (±SD)	**T PROT (±SD)**	**ALB (±SD)**	**T BILI (±SD)**	**AMY (±SD)**	**Ca (±SD)**	**PO4 (±SD)**
Pre	5.9(0.27)	3.6(0.21)	0.2(0.08)	645(84.0)	10.5(0.21)	6.5(0.77)
P-END	4.3(0.51)	2.8(0.41)	0.1(0.04)	362(46.8)	8.5(0.52)	5.9(1.36)
Post Prot/COOL	4.0(0.29)	2.6(0.16)	0.1(0.04)	386(50.5)	8.2(0.08	6.3(1.51)
Post Day 1	5.2(0.32)	3.4(0.17)	0.4(0.16)	416(50.8)	9.7(0.32)	5.7(1.01)
Post Day 4	5.4(0.33)	3.4(0.19)	0.4(0.26)	567(150.1)	10.2(0.29)	6.3(1.22)
Post Day 7	5.2(0.17)	3.4(0.17)	0.2(0.10)	862(103.9)	10.3(0.13)	6.1(0.40)

RESULTS

Six laboratory-raised male dogs were used in this study. One animal acted as the control and was subjected

to the perfusion protocol without undergoing hyperthermia. The remaining five animals underwent EWBH, as stated in the methods portion of this paper. There were no perioperative deaths. All animals were clinically well throughout the study, as assessed daily by a veterinarian. All animals went to completion of the study, including a thorough necropsy.

Table 2: Control equals one animal; values for heated animals are represented as average ± standard deviation

CONTROL	LDH	ALT	AST	ALK PHOS	CK
Pre	55	43	27	49	87
P-END	90	36	26	41	216
Post Prot/COOL	85	34	25	41	209
Post Day 1	666	107	700	151	18392
Post Day 4	75	119	45	99	471
Post Day 7	209	79	29	78	203
AVG ANIMALS 1 – 5 (±SD)	**LDH (±SD)**	**ALT (±SD)**	**AST (±SD)**	**ALK PHOS (±SD)**	**CK (±SD)**
Pre	83(38.80)	29(5.97)	26(3.49)	71(15.42)	125(14.94)
P-END	129(17.85)	23(5.08)	26(4.59)	80(12.99)	131(47.29)
Post Prot/COOL	304(70.71)	24(6.25)	54(12.20)	99(25.95)	318(113.69)
Post Day 1	1585(1264.03)	1032(822.46)	2234(1462.73)	153(76.04)	46417(28150.06)
Post Day 4	65(19.55)	936(874.95)	132(144.80)	139(81.90)	1500(1752.48)
Post Day 7	14(126.08)	501(497.73)	45(21.32)	101(58.00)	284(130.71)

LABORATORY RESULTS

Serum laboratory measurements were assessed at baseline (pre-anesthesia, pre-bypass), multiple intervals throughout the procedure, and at 1,4, and 7 days following bypass.

Biochemical serum profiles revealed a drop in sodium and chloride at the end of extracorporeal circulation in all animals, with a 100% return to normal levels by day 4 post procedure. Potassium levels fell below baseline one day following the experiment, only to return to normal values at day 4. Glucose levels were elevated during the procedure; however, replacement fluid during the study consisted of D5W. Blood Urea Nitrogen (BUN) rose in all animals at the end of the procedure, and three heated animals had further rise in BUN one day following treatment. Elevated BUN levels returned to normal range by day 4 in all animals. Creatinine levels also rose during the procedure, but were normal by day 1 post procedure.

Total protein (TProt) and albumin (Alb) levels dropped during the treatment to lows of 61.3%, 66.7%, respectively, as compared to baseline, and rebounded to normal by day 7 (Table 1). Total bilirubin (TBili) was elevated in the control animal and in one of the hyperthermic treated animals at the end of extracorporeal circulation. Three of the remaining heat treated animals had an elevation of TBili at one day following the procedure. All elevated levels approached normal values by day 7.

Amylase (Amy) levels dropped during bypass with lowest levels seen at the end of bypass. Suppressed levels returned to baseline by day 4 in the control animal and 40% of the heat treated animals. The remaining heated animals had achieved baseline Amy levels by day 7 (Table 1).

Calcium (Ca) levels dropped at the end of the procedure in all animals (control and heated). Phosphorus (PO_4) levels rose in the control animal and in two of the heated animals at the end of bypass. Altered parameters returned to normal levels in all of the animals by day seven (Table 1). Magnesium levels in control and heated levels dropped at the end of bypass; however, there was a more substantial drop in the heated animals. Magnesium levels rebounded to baseline values by day one post procedure.

Table 3: Control equals one animal; values for heated animals are represented as average ± standard deviation

CONTROL	WBC	HGB	HCT	PLT
Pre	13.6	17.1	49.9	208
P-END	8.6	13.2	38.2	175
Post Prot/COOL	9.6	13.2	37.5	160
Post Day 1	30.5	16.0	46.7	182
Post Day 4	14.7	15.4	46.4	244
Post Day 7	14.6	16.4	45.5	242
AVG ANIMALS 1 – 5 (±SD)	**WBC (±SD)**	**HGB (±SD)**	**HCT (±SD)**	**PLT (±SD)**
Pre	10.1(1.50)	16.2(0.81)	47.3(1.82)	283(35.16)
P-END	3.5(1.60)	11.8(3.04)	34.6(8.53)	66(12.42)
Post Prot/COOL	8.0(2.50)	11.8(1.36)	35.3(4.30)	25(13.66)
Post Day 1	42.2(10.48)	16.1(2.06)	47.9(6.63)	11(2.19)
Post Day 4	12.1(4.75)	13.6(1.23)	39.9(3.67)	146(43.31)
Post Day 7	9.5(1.69)	12.5(0.82)	36.5(2.79)	301(43.54)

Lactate dehydrogenase (LDH), alanine transaminase (ALT, SGPT), alkaline phosphatase (Alk Phos), aspartate transaminase (AST, SGOT), and creatinine Kinase (CK) all rose by day one following treatment. ALT, AST, and CK demonstrated a decreasing trend towards pre-procedure levels by day seven, although baseline levels were not reached by the time of necropsy (Table 2). The white blood cell counts (WBC) fell in all animals during extracorporeal circulation only to rise substantially one day following the treatment. An increase in neutrophils and lymphopenia was most pronounced in the hyperthermia treated animals. WBC levels were in the normal range by day 7 in all animals (Table 3). Each animal's hemoglobin (Hgb) and hematocrit (Hct) levels dropped at the end of the procedure, but were within normal limits by the first day following bypass.

Plasma free hemoglobin levels (PFHgb) were elevated from baseline in four of the six animals at the conclusion of hyperthermic perfusion/post protamine administration; however, no level exceeded 9.6 mg/dL in the hyperthermic animals. At day 1 post bypass, 5 of the 6 animals had elevated levels from baseline with only one of these animals having visible evidence of hemolysis. At day 7, no hyperthermic treated canine had a plasma free hemoglobin level greater than 8.0 mg/dL. The control animal demonstrated an elevated plasma free hemoglobin with a value of 7.4 mg/dl following bypass. The first day following the treatment, the plasma free hemoglobin level was 70.0 mg/dl and visibly, the plasma was red tinged. By day 4, the plasma free hemoglobin was 7.8 mg/dl, and on day seven, the plasma free hemoglobin level had risen to 15.5 mg/dl with no visible evidence of hemolysis.

In all animals, platelet levels (Plt) dropped substantially, and, except for the control animal, remained depressed until day 7. Platelet levels returned to baseline in the control animal by 4, and in the heat treated animals, baseline levels were reached at day 7 (Table 3).

Protime (PT), partial thromboplastin time (PTT), thrombin time (TT) and activated clotting time (ACT) levels were all elevated during hyperthermic perfusion. A heparin loading dose and subsequent doses of heparin were required to keep the ACT levels elevated during bypass. Protamine sulfate was given following decannulation, in order to reverse the heparin effect. PT, PTT, TT and ACT all returned to normal by day 1. Fibrinogen levels fell during bypass in the control animal and in 3 of the 5 heat treated canines. These depressed fibrinogen levels rose in 2 of the 3 hyperthermic treated animals by day 1. Normal levels were seen in all of the animals, control and heated, by day 4.

Blood gas analyses were measured to help assess acid/base status. All animals were purposely kept acidotic from the early bypass period to the end of bypass. The following measured values were found in all animals: pH, range 7.31-7.40; pO_2, range 385.4-620.3 mmHg; pCO_2, range 31.0-42.4 mmHg; and base excess, range -1.1--5.1 mmol/L.

NECROPSY

All animals underwent pathological examination. Tissue sampling included gross and microscopic sectioning of the following organs: brain (cerebral cortex, cerebellum), spinal cord, adrenals, liver, spleen, pancreas, kidneys, vena cava, aorta, gastrointestinal tract, thymus, thyroid, parathyroids, lungs, heart, skeletal muscle sampling, and lymph nodes (regional and mesentery).

Gross examination revealed no obvious organ infarcts or necrosis. Histological sectioning supported the gross findings of no infarctions, inflammatory changes, nor evidence of organ infections. At the sites of the surgical incisions in the inguinal areas, there were grossly small anemic or foci of necrosis in the belly of the pectineus muscle in all animals. Histologically, coagulation necrosis with attempts at muscle regeneration was present. Two canines had small foci of hemorrhage in the temporal muscle, unilaterally, and microscopic sectioning demonstrated evidence of coagulation myonecrosis with attempts at regeneration.

Three of the dogs demonstrated minor areas of epicardial hemorrhage in the right atrial appendage. One of the canines showed an isolated area of endocardial hemorrhage in the right atrium, and histological sectioning demonstrated acute ischemic myocardial necrosis. Two animals revealed isolated minor foci of endocardial hemorrhage: one in the left ventricle free wall, and the other in a papillary muscle. Microscopic analysis revealed subacute myocardial cell necrosis and vacuolation of the myocytes. Each animal had normal appearing coronary arteries with no evidence of myocarditis or inflammation.

Two animals demonstrated small areas of subcutaneous/muscle hemorrhage and necrosis in the dorsal aspect of the thorax. Histologically, these areas revealed necrosis with regeneration. A spinal cord lesion was also found in one of these animals, noting a small area of demyelination and axonal degeneration of a dorsal white track, with a few small arterioles containing some fibrin thrombi. Four canines demonstrated splenomegaly at the time of necropsy. Microscopically, splenic congestion with normal appearing erythrocytes were found. All spleens demonstrated no evidence of inflammation, necrosis, infarction, or degenerative changes.

DISCUSSION

Many carcinomas spread beyond their site of origin at the time of diagnosis, by either infiltrating adjacent organs or demonstrating distant metastases. Theoretically, these sites of metastatic involvement arise due to circulating micrometastases, which may become evident in the evolution of a tumor. The presence of this systemic effect either at the time of diagnosis or possibly even earlier in a tumor's differentiation suggests that a more aggressive treatment approach should be considered (4, 5).

It is obvious that WBH can be induced using either a noninvasive or invasive approach. Recently, WBH research in the field of oncology has centered primarily on the use of a noninvasive method, either alone or coupled with radiation and/or chemotherapy (6.7). The toxicity of non-invasive WBH techniques have been projected as within clinically acceptable limits; however, this has not been the case with EWBH. Until new technology and treatment techniques could show a comparable toxicity level to noninvasive WBH, EWBH had all but been abandoned. Serious investigation into EWBH toxicity, and potential efficacy, has been lacking, because a reasonable animal technique did not exist. Therefore, EWBH has not received ongoing evaluation in a pre-clinical setting.

The literature has reported only scant investigations into the use of EWBH involving modified dialysis. Either the placement of an arteriovenous shunt or a patient's native artery and a vein were connected to the dialysis device. The use of an artery as part of the circuit is not without its potential complications. Arterial cannulation can cause complications during and follow-

ing the procedure, such as arterial dissection, "steal syndrome," distal organ ischemia/infarction, and possible emboli. In 1994, Alonso, et al., reported results of long term follow up in 31 HIV patients treated with low flow, arterial-veno bypass involving the use of a roller pump. Two of these patients died of treatment related complications, cardiac arrhythmia and CNS, respectively (8). The technique described in this paper utilizes two veins, thereby alleviating potential arterial complications. A small diameter artery used in any circuit could compromise the effective lumen of the vessel following the repair of the vessel at the conclusion of the procedure. Our veno-venous approach centers on the generous compliance property of a systemic vein and therefore size requirement with potential restrictiveness following any repair is less of a concern.

Our low flow technique using an independent flow control centrifugal pump encountered no physiological difficulties during either the heating or plateau phases. Physiological instability requiring fluid replacement and electrolyte adjustments has been reported with high flow bypass techniques in inducing WBH. Parks, et al., in using a modified arterio-venous high flow technique, did report serum deficiencies of potassium, magnesium, and phosphorus during and following EWBH (2).

Subjectively, other patients experienced muscle weakness and findings of pulmonary edema (4). Our technique did not result in significant phosphorus or magnesium deficiencies, or in neurologic, muscular, or pulmonary problems. These findings are similar to both Page's and Macy's findings in canine treated tumor and non-tumor baring dogs utilizing non-invasive devices (9, 10).

The observed minor pathological changes in this study can be justified. The placement of cannulae or the Swan-Ganz catheter may have caused the minor trauma to the endocardial surface. No animal experienced any cardiovascular compromise during the procedure or throughout the follow up period. Splenomegaly with congestion is a common finding at post mortem examination following induction of anesthesia. No pathological abnormalities were found with these enlarged spleens. Some animals hit the sides of their head against the holding cage during recovery and sustained trauma to the temporal muscle areas. By not housing the remaining animals in these cages during the "surfacing period" from anesthesia, this problem was eliminated. The surgical inguinal cutdowns performed in isolating the desired vessels for cannulation carries potential problems to adjacent muscles/tissues in that area if blood flow is interrupted. Interruption of small vessels during isolation of each vessel can impede flow to a muscle belly supplied by that vessel. However, long term compromise was negligible.

Biochemical assessment comparing our technique to others revealed results similar to non-invasive technologies. WBH produces laboratory abnormalities. The observed electrolyte abnormalities reverted back to baseline within the first week post treatment. However, this was not the case in the assessment of liver function. Page, et al., reported significant increases in alkaline phosphatase 24 hours post WBH using a non-invasive device in tumor bearing dogs (9). The marked elevation seen in liver enzymes during our study mirrored their results with baseline values or a trend towards baseline usually seen by day 7 of follow up. Similarly, WBC levels, including differentiation, revealed alteration with the induction of WBH by Page, Macy, and by our group (9, 10). While absolute WBC counts were depressed and marked granulocytosis and lymphopenia were observed in our animals, the non-invasive methods showed an increase in WBC during this same time frame. Return to baseline occurred within the first week post treatment in all reports. Unlike non-invasive, EWBH markedly stressed platelet levels during and following the procedure. The Page group reported a mild platelet depression observed 24 hours post WBH, but these depressed values were still within the hospital reference range (9). However, the significant depression we saw in platelet count rebounded within the first four days following the insult with no bleeding tendencies.

In summary, the new Organetics, Ltd. PS-1 technology demonstrated safety and effectiveness in assessing the bypass device and EWBH treatment. Many questions concerning toxicity of using this technology for extracorporeal induction of hyperthermia were answered. The canine was demonstrated to be an effective model with acceptable toxicity in providing the means to investigate the potential merit of invasive whole body hyperthermia as a therapeutic option in an array of systemic diseases.

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